Effects of legume growth and residue decomposition on growth and phosphorus uptake in following wheat

A thesis submitted to The University of Adelaide in fulfilment of the requirements for the degree of Doctor of Philosophy.

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Dedicated to my father, Mat Hassan Mohamad and mother, Wan Limah Wan Abdullah

Table of Contents

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Abstract

In phosphorus (P) deficient soils, several legumes have been shown to mobilise less labile P pools and to have a greater capacity to take up P than cereals. In conditions where N was not limiting, some legumes can increase the growth and P uptake of the following cereals which may be related to P mobilisation by the legumes. There is little information about the size of various soil P pools in the rhizosphere of legumes in soil fertilised with P although P fertiliser is often added to legumes to improve N_2 fixation. The aims of this study were to (i) compare the growth, P uptake and the concentration of rhizosphere soil P pools of different grain legumes, (ii) compare the decomposition rate of grain legume and wheat residues, and (iii) determine the effect of legume pre-crops and residue addition on growth, P uptake and concentrations of rhizosphere P pools of the following wheat.

A series of plant growth experiments were carried out in a glasshouse to compare the growth of the different grain legumes and wheat and the concentrations of P pools of the rhizosphere soil. The soil pH determines the dominant P forms, therefore, two soils which were low in available P and contrasting pH (a loamy sand soil pH 8.8 and a sandy loam pH 5.4) were used in separate experiments to which soluble P was added to ensure good plant growth. Additionally, another experiment was conducted in the alkaline soil with lower P supply. Nodulated chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* L.), white lupin (*Lupinus albus* L.), yellow lupin (*Lupinus luteus* L.) narrow-leafed lupin (*Lupinus angustifolius* L.) and wheat were grown until maturity. Plant dry weight and P uptake were measured, sequential P fractionation was employed to determine the concentrations of P pools in the rhizosphere of the legumes and wheat.

Irrespective of soil pH and P supply, growth and P uptake were greatest in faba bean whereas the less labile P pools were most strongly depleted in the rhizosphere of white lupin despite its lower growth and P uptake compared to faba bean. In the alkaline soil with high P supply, compared to the unplanted control soil, the depletion of labile pools (resin P and

$NaHCO_3$) were greater in the rhizosphere of faba bean whereas in the alkaline soil with low P supply and the acidic soil, white lupin depleted most of the labile pools more strongly than the other legumes.

An incubation study was carried out to compare the decomposition rate and the available N and P concentrations after addition of the legume and wheat residues. Shoots, roots and the combination of shoots and roots of wheat, faba bean, chickpea and white lupin were mixed into the loamy sand soil. The decomposition rate was measured over 42 days by determining soil CO_2 release and the concentrations of available P and N in the soil were measured on days 0 and 42. Chickpea shoot residue decomposed faster than the other residues. Compared to the control soil without residue addition, resin P concentration was increased with legume residue addition but not with wheat residue addition. Inorganic N was increased significantly with addition of faba bean and white lupin residues compared to the un-amended control whereas wheat residue addition had no effect.

In order to differentiate between the effect of the legume pre-crop alone and that of legume pre-crop and their residue on the following wheat, soil grown with legumes from which root and shoot residues were removed or added back were planted with wheat. Growth, P uptake and concentrations of rhizosphere P pools of the following wheat were measured. Generally, growth was greater in wheat grown in the previously unplanted soil than in the pre-cropped soils. Among the pre-crops, in the alkaline and acidic soils with high P supply, the growth of the following wheat was greater in legume pre-crop soil without residue than with residue addition. The reverse was true for plant P concentration in the alkaline soil whereas in the acidic soil, plant P concentration was similar among the treatments. Varying results with residue addition on the growth of following wheat were observed in the alkaline soil with low P supply, but residue addition consistently increased wheat P concentration. In the loamy sand (pH 8.8) with high P supply, regardless of the pre-crops, wheat depleted the less labile residual P, NaOH-Pi and particularly NaOH-Po, whereas in the sandy loam (pH 5.4), the

depletion was greatest in resin P. Similarly, in the loamy sand soil with low P supply, wheat after legumes depleted labile and less labile pools more than wheat after wheat. Generally, the addition of pre-crop residues increased the size of organic P pools in the rhizosphere of wheat grown in pre-crop soils.

The results of this study showed that in the alkaline loamy sand, among the legumes only those with the greatest depletion of either labile or less labile pools (faba bean at high P and white lupin at low P supply) enhanced the growth of the following wheat. At high P supply, the pre-crop faba bean with greatest depletion of labile pools resulted in a greater depletion of less labile pools by the following wheat than the other legumes. At low P supply, the pre-crop white lupin with greatest depletion of labile and less labile pools induced a greater depletion of the less labile pools in the rhizosphere of wheat. On the other hand, in the acidic sandy loam, the legumes with the greatest depletion of most pools (labile and less labile) did not increase the growth of the following wheat compared to legumes with little depletion. Furthermore, the addition of legume pre-crop residues increased the concentration of organic P pools in the rhizosphere of the following wheat compared to pre-crop alone but generally decreased wheat growth.

Declaration

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Hasnuri Mat Hassan

Chapter 1

Introduction and review of literature

1 Introduction and Review of literature

1.1 Introduction

Phosphorus (P) is the second most important nutrient after nitrogen for all living organisms. It is an essential component for the organic compound such as adenosine triphosphate (ATP), deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and phospholipids which play key roles in energy metabolism, storage and transfer of genetic information and as structural components in the cell (Brady and Weil 2002; Lavelle and Spain 2002). In the soil, P is found in different forms/pools which vary in availability to plants. Phosphorus deficiency can limit plant growth and production and farmers tend to apply P fertilisers in excess of plant requirement to sustain crop production. This has resulted in a build-up of less labile inorganic P and residual P in the soil (Wang et al. 2007b; Vu et al. 2008) and runoff this element can increase the risk of eutrophication in nearby water bodies (Sharpley 1995; Sharpley et al. 2000).

Wheat is a staple food in many countries of the world and the most important grain crop in Australia. In 2002, wheat was grown on more than 240 million ha worldwide which was larger than for any other crop (FAO 2002). World wheat production is often limited by nutrient deficiency particularly N and P. Legumes (grain and pasture) have become important crops in farming systems due to their beneficial effects on soil N and P availability as well as maintaining soil health in crop rotations (Armstrong et al. 1997; Asseng et al. 1998; Nuruzzaman et al. 2005a). Apart from the capacity to fix atmospheric N_2, legumes have been shown to have greater capacity for P uptake than cereals and mobilise P from poorly available soil P pools. Several previous studies demonstrated the ability of legumes to increase growth and P uptake in the following cereals (Horst et al. 2001; Kamh et al. 2002; Nuruzzaman et al. 2005b, a). These positive effects of legumes are proposed to be due to P mobilisation by root exudates during legume growth (Nuruzzaman et al. 2005b, a), release of P from the decomposing residues (Horst et al. 2001) and P mobilisation during the decomposition of

legume residues (Kamh et al. 2002). However, the relative importance of these mechanisms is not clear.

Legumes may also change the size of the various soil P pools or their availability to the following wheat, however information about the effect of legumes as pre-crops on the changes in the soil P pools of the following crops is scarce. The aim of this review is to provide an overview on the role of the legumes as pre-crops and the effect of their residues on the following crops with respect to crop growth, P uptake and rhizosphere P pools.

1.2 Phosphorus

1.2.1 Phosphorus in the soil

In the soil, P becomes available through several processes including organic matter decomposition, turnover of the microbial biomass, weathering of parent material, and mobilisation of P salts or adsorbed P (Lavelle and Spain 2002). Soil total P comprises various pools of inorganic and organic P forms (Marschner 2008)(See Figure 1 for details of the P cycle). Although the concentration of soil total P is high for example in Australian soil; typically >250 mg P kg^{-1} soil in the top 10 cm (Palm et al. 1997), only a small proportion is immediately available for plant P uptake. According to Conyers and Moody (2009) the concentration of P in the soil solution is low (less than <5 μM). The concentration of Pi in the soil solution which is readily available to plants is maintained by solubilisation of poorly available inorganic P, mobilisation of adsorbed P, mineralisation of organic P (P_o) from soil organic matter, decomposition of plant residues and turnover of microbial biomass. Plant roots absorb P mainly as orthophosphate anions which occur as dihydrogen phosphate ion ($H_2PO_4^-$) in acidic soils and as hydrogen phosphate ion (HPO_4^{2-}) in alkaline soils (Brady and Weil 2002).

Soil inorganic P occurs in soil as minerals, adsorbed and precipitated and it is estimated that 50 to 200 kg P ha^{-1} of soil total P were in inorganic P forms (Richardson 2001). There are

more than 170 different P minerals in the soil and these forms vary greatly in terms of their solubility and transformations from less labile to labile forms (Holford 1997). Stable P pools are primary P minerals including apatites, strengite and variscite and secondary P minerals including P associated with Al, Fe and Ca (Oelkers and Valsami-Jones 2008). In acidic soils, Al- and Fe-phosphates are the predominant forms while in alkaline soils P is likely to occur as Ca- or to a lesser extent as Mg-phosphates (Holford 1997; Brady and Weil 2002). Soil physical and chemical properties greatly influence the forms and solubility of P namely soil pH, concentrations of Fe, Al and Ca and surface areas of the soil particles. Further, inorganic P ions can be absorbed to clay minerals (Fe and Al-hydrous oxides), sesquioxides, and organic matter complexes or can be precipitated with positive charged minerals in the soils (Holford 1997).

Organic P (Po) is an important source of P for plant uptake particularly in tropical soils and constitutes 20% to 80% of total P in the soil (Dalal 1977; Schachtman et al. 1998; Brady and Weil 2002) with the concentration being positively correlated with soil organic matter content. Organic P in soils exist either in stable form as inositol phosphates and phosphonates, and/or labile form as orthophosphate diesters, labile orthophosphate monoesters, and organic polyphosphates (Turner et al. 2002; Condron et al. 2005). Mineralisation of Po into Pi occurs via hydrolysis of phosphate ester (C-O-P), phosphoanhydride (P-O-P) and phosphonate (C-P) bonds (Richardson et al. 2005). The process is mediated by soil microorganisms or plant roots via release of phosphatase enzymes and influenced by soil pH and redox potential, soil moisture, temperature, physical chemical and surface properties (Shen et al. 2011). Previously, the majority of organic P in the soil was thought to be in the form of phytate (inositol hexakisphosphate) (Turner et al. 2002), however it has been challenged recently by Smernik and Dougherty (2007). In their study, they found that in different Australian pasture soils, phytate comprised <5% of organic P and <3% of soil total P.

The soil microbial biomass is considered another important source of organic P which plays a significant role in soil P cycle. Soil microbial biomass is predominantly composed of bacteria and fungi, but also includes algae, protozoa and nematodes (Jacobsen et al. 2005). Microbial biomass P represents about 3% of soil organic P in arable soil and 5 to 24% in grassland soil (Brookes et al. 1984). In the rhizosphere, the microbial biomass takes up available P thus competing with plant roots but may also turnover and then become the source of available P for plant uptake (Seeling and Zasoski 1993; Oberson et al. 2001).

Organic P dynamics are influenced by the interactions among microbes, organic matter and plants in processes such as mineralization, immobilisation and redistribution (Stewart and Tiessen 1987). Mineralisation is the transformation of organic P into inorganic forms while immobilisation is P uptake by the microbial biomass which decreases the concentration of Pi in the soil solution. Both mineralisation and immobilisation of P can occur simultaneously (Dalal 1977; Stewart and Tiessen 1987). Redistribution of P occurs when immobilised P is released or mineralised P immobilised.

The fate of added Pi fertiliser is governed by the P concentration in the soil, size and activity of microbes, organic matter content, P adsorption capacity, time and other soil properties such as pH, moisture, temperature and clay content (Blair and Boland 1978; Iyamuremye et al. 1996; Nziguheba et al. 1998; Nziguheba et al. 2000; Pierzynski et al. 2005)

When P fertiliser is applied, it rapidly becomes unavailable to plants due to adsorption and formation of poorly available P (Wang et al. 2007b; Vu et al. 2008; Vu et al. 2010) with the magnitude of these processes being influenced by soil pH and minerals present in the soil. Plants take up about only 45% of applied P fertiliser during the first year of cropping (Mózner et al. 2012) and the remaining proportion will be adsorbed or precipitated into poorly available forms (Vu et al. 2008). Therefore, farmers have to apply excess P fertiliser to sustain high crop production. Long-term P fertiliser addition in cropping system has resulted in a build-up of the soil P bank which consist of various poorly available P pools (Wang et al.

2007b; Vu et al. 2008; Rose et al. 2010). In Australia, it has been estimated that this soil P bank is equivalent to $5-10 billion worth of P fertilisers (Stevens et al. 1997). Additionally, P fertiliser which is derived from phosphate rock is a non-renewable resource and high quality rock P reserves are expected to run out within the next 50 to 100 years (Cordell et al. 2009). Thus, improvement of soil P availability and utilisation efficiency by crops particularly of the poorly available P forms are important to reduce the reliance of P fertiliser would save farmers substantial amounts of money and make farming more environmental friendly.

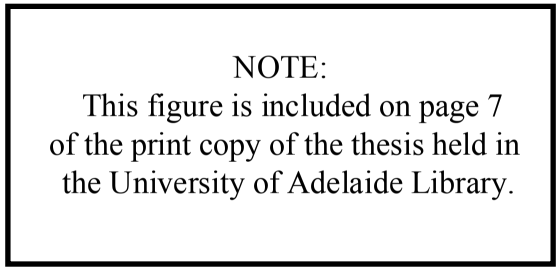

Figure 1: P dynamics in the soil/rhizosphere - plant continuum. C-P, Carbon - P; NO, nitric oxide; OA, organic acids [adapted from Shen et al. (2011)].

1.2.2 Characterisation of soil P

There are several methods that have been developed for the determination of soil total P; the most commonly used methods are sodium carbonate (Na_2CO_3) fusion (Jackson, 1958), perchloric acid ($HClO_4$) digestion (Jackson 1958), sulphuric acid-hydrogen peroxide-hydrofluoric acid (H_2SO_4-H_2O_2-HF) digestion (Bowman 1988), and sodium hypobromite (NaOBr) oxidation followed by H_2SO_4 dissolution (Dick and Tabatabai 1977). In these methods inorganic P is solubilised and organic P is converted into inorganic P; therefore P measured equals total P (Kuo 1996).

Various methods were used to measure available P in the soil. In the early 1950's, Bray P (Bray and Kurtz 1945) and Mehlich 1 (Mehlich 1953) methods were introduced for acidic and neutral soil to extract acid soluble P forms, Al- and Fe- associated P. Mehlich 1 has been modified to suit a wider range of soil pH (Mehlich 1984). A year later, Olsen et al. (1954) introduced a method which uses 0.5 M $NaHCO_3$ solution (at pH 8.5) to extract Ca associated P and adsorbed P onto Fe-oxide surfaces in alkaline soil. The Olsen method was further improved by Colwell (1963) by prolonging the shaking time from 30 minutes to 16 hours; these two methods are widely used for soil P test across Australia. More recently, measurement of available P using anion exchange membranes (resin P) was introduced by Kouno et al. (1995). This method is also suitable for various soil types. Isotopic dilution technique for measuring exchangeable P (E-value) (Morel and Plenchette 1994) and the employment of Diffusive Gradient in Thin films (DGT) (McBeath et al. 2007) are increasingly gaining interest as alternative soil P tests. However, a study showed that the resin P method could better predict P fertiliser responsiveness of wheat than the other common soil P methods (Bray P, Olsen P, Colwell P, E-value and DGT) (McBeath et al. 2007).

Measurement of organic P in the soil is difficult due to its low reactivity with colorimetric compounds. However, a method of soil extraction with NaOH-EDTA followed by ^{31}P nuclear magnetic resonance (NMR) spectroscopy was able to provide more details on the

characterisation of not only soil organic P but condensed P forms (Turner et al. 2003; Bünemann et al. 2008).

Although P pools are conceptual, P fractions are chemically defined. Sequential P fractionation is widely used to assess the size of the various P pools including organic and inorganic forms which differ in availability. The method has been regarded as an important tool in the study of P biogeochemistry for the past 50 years (Condron et al. 2005; Pierzynski et al. 2005).

Various P fractionation schemes were developed to characterise different P forms in soils and sediments (Chang and Jackson 1957; Bowman and Cole 1978; Hedley et al. 1982; Tiessen and Moir 1993). The underlying basis of the methods is using a series of extractants sequentially, with mild extractants to remove labile P, then less available or more stable form of P can be extracted by stronger acids and alkali. In the method developed by Hedley et al. (1982), microbial P was included in the procedure considering its significant role for soil P cycling (Cole et al. 1977). However, this fractionation procedure left 20 to 60% of soil total P. Therefore this method was further improved by Tiessen and Moir (1993) (Figure 2). In the extraction scheme, resin extractable P represents the most labile pool that originates either from soil solution or weakly absorbed P onto hydroxides or carbonates. Bicarbonate extractable P (0.5 M $NaHCO_3$, pH 8.5) also represents weakly sorbed P (Pi) and easily hydrolysable organic P (Po) which equates to plant-available P (Hedley et al. 1982; Tiessen and Moir 1993; Linquist et al. 1997). The P in the Pi in the 0.1 M NaOH is associated with amorphous and crystalline Al and Fe phosphates and clay minerals, and Po is associated with fulvic and humic acid, which are stable forms of organic P. The diluted HCl pool represents P associated Ca (apatite and octacalcium) while P in a more stable pool is extracted by the hot concentrated HCl extract. Residual P (after the hot concentrated HCl extraction) is considered as the most recalcitrant P pool (Tiessen and Moir 1993). Generally, the availability of P pools for plant uptake decreases with increasing strength of the extractant in the fractionation

scheme, however the availability also depends on several other factors including root growth, P diffusion and dissolution, soil properties e.g., pH and fertiliser history.

There is no direct measurement of organic P for each fraction; hence Po is calculated by the difference of total P and inorganic P. Although the determination of total P is quite reliable, Pi determination may overestimate inorganic P due to incomplete precipitation of organic matter particularly in bicarbonate and hydroxide extracts (Tiessen and Moir 1993). The dynamic of the P pools is complex and influenced by soil type and other environmental conditions (Guo et al. 2000; Wang et al. 2007b). In an unfertilised alkaline soil, acid-soluble and residual P pools were depleted suggesting that these pools act as buffer to replenish plant available P (Wang et al. 2007). On the other hand, Beck and Sanchez (1994) showed that hydroxide extractable Pi (NaOH-Pi) was closely related to plant available P in an 18 years cultivated and fertilised soil. Phosphorus added with fertiliser preferentially accumulated in labile inorganic and moderately labile pools (Wang et al. 2007b; Vu et al. 2008) thus increasing soil fertility.

A considerable number of studies have shown differential ability of various crops to access different P pools (Wang et al. 2008b; Rose et al. 2010; Wang et al. 2010). Generally, the labile pools are readily available for plant uptake; however the accessibility of the less labile pools differed among plant species. Significant depletion of organic and inorganic P of the less labile pools by white lupin was attributed to the exudation of organic acid anions and phosphatases by its cluster roots (Braum and Helmke 1995; Kamh et al. 1999; Nuruzzaman et al. 2006; Wang et al. 2008b). In another study, species that excreted extracellular phytase depleted the organic hydroxide extractable P (NaOH-Po) in their rhizosphere (George et al. 2006). Moreover, a study by Vu et al. (2008) showed that both wheat and chickpea were able to deplete most P pools in a calcareous soil. The inconsistent results of accessed P pools by various plant species indicate that soil properties (mineralogy and pH) as well as adaptive

mechanisms by specific species can have a large impact on accessibility of various P pools and its dynamics.

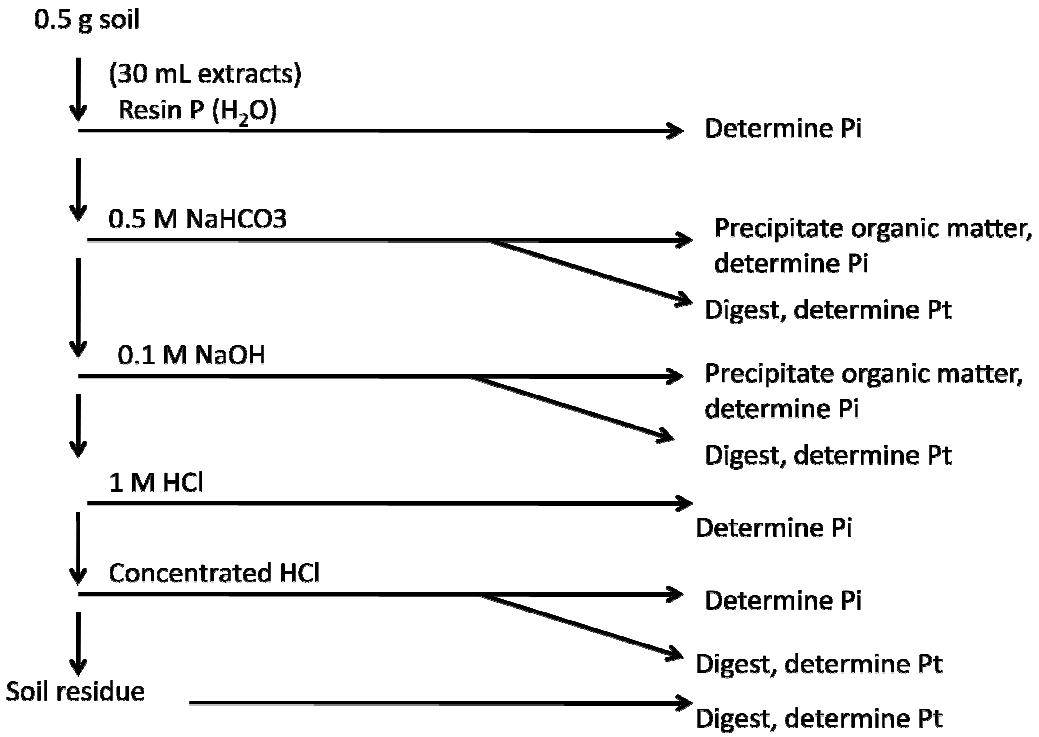

Figure 2: Sequential P fractionation of Tiessen and Moir (1993)

1.3 Phosphorus uptake, mobilisation and mineralisation

Phosphorus uptake by plants is determined by P availability in the soil and plant properties (Föhse et al. 1988). According to Barber (1995), P acquisition is limited by the slow movement of P to the plant root which results in rapid depletion of available P in the rhizosphere due to root activity (Jungk and Claassen 1986). Plants acclimatise to low P availability by developing various morphological, physiological and biochemical changes to acquire P (Lambers et al. 1998). In summary, morphological changes are the modification of root growth and architecture to maintain the capacity to explore a larger soil volume whereas physiological and biochemical changes are related to changes in the internal and cellular metabolism of P as well as root activities which affect the availability of P in the rhizosphere (Tinker and Nye 2000; Vance et al. 2003; Richardson et al. 2009).

Development of more extensive root systems and root hairs, increasing root length and root/shoot ratio are among the morphological adaptations of plant roots to enhance the soil volume accessed by the plant (Föhse et al. 1988; Bates and Lynch 1996; Gahoonia and Nielsen 1998). A study conducted in Western Australia showed that faba bean with a greater root biomass had greater P uptake than other legumes and wheat (Nuruzzaman et al. 2005a). Some plant species modify their root structures to increase specific root length (SRL) and consequently increase the surface area for P acquisition. Plant achieved higher SRL by having more branches roots per unit root dry matter (Hill et al. 2006) and reducing root mass density (Fan et al. 2003) and root diameter (Hill et al. 2006). In addition, white lupin and some species of family Proteaceae are well-known for the formation of specialised root structures; cluster roots or proteoid roots (Neumann et al. 1999; Neumann et al. 2000; Shen et al. 2003; Hocking and Jeffery 2004; Li and Liang 2005; Shen et al. 2005; Pearse et al. 2006b; Wang et al. 2006; Wang et al. 2008a). These specialised root structures can make up to 40% of root dry weight under P deficient conditions (Dinkelaker et al. 1989) which mobilise P by root exudates (carboxylates and phosphatase).

It is well established that nutrient deficiency (Fe, Zn and particularly P) and Al toxicity stimulate exudation of carboxylates by cluster and non cluster-rooted species. Carboxylates are low-molecular-weight organic acid anions include tricarboxylates (citrate), dicarboxylates (fumarate, malate, oxalate and malonate) and monocarboxylates (acetate) where the effectiveness of these compounds to mobilise soil P depends on the number and arrangements of carboxyl and hydroxyl groups (Ryan et al. 2001). The mechanisms for mobilising P are suggested due to: (i) reducing the number of binding sites for P fixation via chelation of Ca, Fe and Al (Gerke 1992; Jones and Edwards 1998) and (ii) replacing/competing with phosphate for adsorption sites in soil and P-fixing minerals (Nziguheba et al. 2000; Ryan et al. 2001). The composition and proportion of the carboxylates may vary with crop species and soil types (Veneklaas et al. 2003; Wouterlood et al. 2004; Nuruzzaman et al. 2006).

Another universal response to soil P deficiency is the exudation of phosphatase enzymes to mineralise organic P into inorganic forms (Jungk et al. 1993; Nuruzzaman et al. 2006; Richardson et al. 2009). In addition, phosphatase enzymes may also be derived from microorganisms (Richardson et al. 2001) The enzyme activity in the soil depends on soil type, plant species and age (Tarafdar and Jungk 1987). George et al. (2006) showed that the depletion of the NaOH-Po pool was positively correlated with the activity of acid phosphatase in the rhizosphere of tithonia and transgenic clover however the degree of mineralisation is also influenced by the stability of organic P pools and microbial activity (Bowman and Cole 1978).

Many studies have demonstrated the modification of the rhizosphere pH as another adaptive mechanism to overcome P deficiency (Bagayoko et al. 2000; Hinsinger 2001; Neumann and Römheld 2002). Modification of the rhizosphere pH is modulated by extrusion of H^+ or OH^- ions and the process depends on various factors such as soil buffering capacity, soil moisture level and aeration, CO_2 production by plant roots and microorganisms, microbial acid production, root exudation of carboxylates, plant genotype, and nutritional status of the plant. However, cation/anion uptake ratios and N assimilation are considered the most important factors for the process (Neumann and Römheld 2002). Preferential uptake of nitrogen as NO_3^- leads to excess uptake of anions and consequently the removal of protons, which increases the pH, whereas excess uptake of cations over anions due to NH_4^+ uptake leads to proton extrusion thus decreasing the rhizosphere pH. In acidic soils, rhizosphere alkalisation may enhance P availability by releasing P adsorbed onto Fe and Al oxides (Gahoonia and Nielsen 1992; Jungk et al. 1993) whereas rhizosphere acidification may enhance the solubilisation of acid soluble Ca-phosphates in neutral and alkaline soils (Gahoonia and Nielsen 1992).

Mycorrhizal fungi, which can explore a greater soil volume by their extensive hyphae network (Smith and Read 2008), are known for their role in enhancing P uptake by plants.

However, the role of mycorrhiza in P uptake in agriculture is questionable as modern cereals cultivars generally have low mycorhhizal responsiveness (Zhu et al. 2001) and soil disturbance such as tillage can destroy the hyphae network (Jasper et al. 1989).

The soil microbial biomass, which represents between 1 and more than 10% of the total soil P (Richardson 2001), is another factor which affects P mobilisation. Their activity is stimulated by an increase in available carbon (De Nobili et al. 2001) either from residues or root exudates from the plant roots. The mechanisms of P mobilisation are similar to those of plants, namely acidification and/or excretion of organic acid anions to mobilise inorganic P forms (Illmer and Schinner 1992; Illmer et al. 1995) and mineralisation of organic P by excretion of phosphatase and phytase (Richardson and Hadobas 1997; Richardson 2001). However, the microbial biomass can be more competitive for P (McLaughlin and Alston 1986), and thus capable of reducing P uptake by plants. When residues with high C/P ratio are added to a soil, P may be immobilised by the microbial biomass; however, when C concentration decreases, the decomposition of dead microbial cells become the source of available P (Seeling and Zasoski 1993; Oberson et al. 2001; Marschner et al. 2011).

The capacity to solubilise or mineralise sparingly available P may differ among microbial species. Therefore microbial community composition in the rhizosphere which differs considerably among plant genotypes (Marschner et al. 2001) may also contribute to the differential ability of plants to take up P. Furthermore, as mentioned above, plant root exudates could favour rapid microbial turnover and stimulate rhizosphere microorganisms with high P mobilisation capacity (Marschner et al. 2006).

1.4 Phosphorus mobilisation and P uptake by legumes

Legumes play an important role in agricultural systems as, unlike other crops, they are capable of fixing nitrogen from the atmosphere. Grain and forage legumes are grown on 180 million ha worldwide and the demand for legume production for dietary protein is expected to increase (Vance 2001).

Nitrogen fixation results in acidification of rhizosphere soil (Tang et al. 1998; Hinsinger et al. 2003) which increases P availability in neutral and alkaline soils (Vu et al. 2008) via solubilisation of calcium phosphates below pH 7 (Hinsinger 2001; Hinsinger et al. 2003). In an acidic soils, preferential uptake of nitrate by plants leads to increased P availability due to solubilisation of Al- and Fe-bound P (Rose et al. 2010).

Legumes have a greater capacity to mobilise P from poorly available P than cereals (Kamh et al. 1999; Nuruzzaman et al. 2005a, b), however this ability is species and soil type dependent. Apart from the modification of the rhizosphere pH, the ability to mobilise P particularly of the less labile pools is due to the secretion of carboxylates such as malate and citrate (Sas et al. 2001; Shen et al. 2003; Veneklaas et al. 2003; Nuruzzaman et al. 2006; Pearse et al. 2006b; Wang et al. 2008a) and acid phosphatases (Gilbert et al. 1999; Li et al. 2004; Jemo et al. 2006; Nuruzzaman et al. 2006; Wang et al. 2007a; Ma et al. 2009).

In a glasshouse experiment, the yield increase with increasing amount of applied P fertiliser was less in legumes than in wheat and canola (Bolland et al. 1999). This was attributed to the ability of these legumes to utilise adequate P from the soil. The greater growth and P uptake of legumes compared to wheat can be explained by the release of root exudates by white lupin and field pea or the extensive root systems in faba bean (Nuruzzaman et al. 2005a, b). In summary, legumes are efficient at acquiring P from various P pools due to extensive root systems (Nuruzzaman et al. 2005b) and the formation of cluster roots (Shen et al. 2003; Wang et al. 2008a) as well as the ability to modify rhizosphere pH (Hinsinger 2001; Tang et al. 2004) and to exude a significant amount of carboxylates and phosphatases (Li et al. 2003; Li et al. 2004; Pearse et al. 2006a).

1.5 Legume effects on the following cereal

Several studies have shown that some legumes increased growth and P uptake of the following cereal crop (Vanlauwe et al. 2000; Carsky et al. 2001; Nuruzzaman et al. 2005b) as well as that of intercropped cereals (Ae et al. 1990; Li et al. 2003; Li et al. 2004; Wang et al.

2007a; Li et al. 2008). In a rotation study with different soils of Western Australia, faba bean had a greater positive effect on growth and P uptake of the following wheat than white lupin and field pea (Nuruzzaman et al. 2005a).

It has been suggested that the exudation of carboxylates from legume roots can mobilise P for the following cereals (Kamh et al. 1999). However, this has been challenged due to the rapid decomposition of carboxylates by soil microoganisms (Nuruzzaman et al. 2005a, b). Therefore a beneficial effect of carboxylates is more likely in inter-cropping systems where the roots of both plants intermingle.

The increased cereal growth after legumes has also been explained by P recycling through legume crops (Horst et al. 2001; Kamh et al. 2002). The release of P from decomposing legume residues could contribute to the enhanced growth and P uptake in the following cereals (Nziguheba et al. 2000). Legumes had greater capability of utilising soil P than cereals (Bolland et al. 1999; Nuruzzaman et al. 2005b; Pypers et al. 2007) therefore it is expected that legume residues contain more P than cereal residues.

Decomposition of crop residues is influenced by microbial activity, residue quality (phenol-protein complexes, initial C:N and C:P ratio, lignin and humic concentration) and environmental conditions such as moisture and temperature (Lavelle and Spain 2002). In addition, mineralisation and immobilisation of P is influenced by the C/P ratio of the added residues. Crop residues with a C/P ratio less than 300 are likely to induce net P mineralisation while net immobilisation is likely at C/P >300 (Cheshire and Chapman 1996; Brady and Weil 2002). Furthermore, residues could increase P availability by microbial metabolites such as carboxylates and phenolic compounds released during the decomposition which can mobilise P and reduce the P fixation capacity of the soil (Hu et al. 2005a; Hu et al. 2005b). In an acidic soil, addition of pearl millet residues resulted in increasing soil pH and base saturation, thus decreasing the exchangeable Al (Hafner et al. 1993), thereby reducing P adsorption.

1.6 Aim of this study

Previous studies suggested that the enhanced growth and P uptake of following wheat may be due to legume growth and/or decomposition of legume residues. However, the mechanisms by which soil P pools are mobilised and how this is affected by residues are not well understood. In particular, there are limited numbers of studies about which soil P pools are accessed during legume-wheat crop rotations and whether the capacity of the following cereals to mobilise P is due to legume-induced changes or to the presence of legume residues. Therefore, the objective of this study is to characterise the changes in rhizosphere P pools in legume pre-crops and the effect of the pre-crop residues on growth, P uptake and the size of rhizosphere P pools of the following wheat. The focus of this study was the rhizosphere because any changes in P pools induced by the roots will be greatest in this soil compartment and it is also the source of P uptake by the plants.

To fulfil the objective, the study specifically aims to:

- Quantify the growth of five grain legumes grown in an alkaline soil with high P supply as well as to compare their P uptake and the changes in the rhizosphere P pools (Chapter 2).
- Compare wheat and pre-crop residue quality (decomposability and availability of P and N) and to assess the effect of pre-crop legumes either in the presence or absence of residues on growth, P uptake and concentrations of P pools in the rhizosphere of the following wheat in an alkaline soil with high P supply (Chapter 3).
- Determine growth, P uptake and the size of the rhizosphere P pools in the legume pre-crops and the following wheat in an acidic soil and how the pre-crop effect is modulated by residue addition (Chapter 4).

- Assess the effect of pre-crop legumes and their residues on growth, P uptake and concentrations of P pools in the rhizosphere of the following wheat in an alkaline soil with low P supply (Chapter 5)

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Chapter 2

Growth, P uptake in grain legumes and changes in rhizosphere soil P pools

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Growth, P uptake in grain legumes and changes in rhizosphere soil P pools

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Abstract

In soils with low P availability, several legumes have been shown to mobilise less labile P pools and a greater capacity to take up P than cereals. But there is little information about the size of various soil P pools in the rhizosphere of legumes in soil fertilised with P although P fertiliser is often added to legumes to improve N_2 fixation. The aim of this study was to compare the growth, P uptake and the changes in rhizosphere soil P pools in five grain legumes in a soil with added P. Nodulated chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* L.), white lupin (*Lupinus albus* L.), yellow lupin (*Lupinus luteus* L.) and narrow-leafed lupin (*Lupinus angustifolius* L.) were grown in a loamy sand soil low in available P to which 80 mg P kg^{-1} was added and harvested at flowering and maturity. At maturity, growth and P uptake decreased in the following order: faba bean > chickpea > narrow-leafed lupin > yellow lupin > white lupin. Compared to the unplanted soil, the depletion of labile P pools (resin P and $NaHCO_3$-P inorganic) was greatest in the rhizosphere of faba bean (54 and 39%). Of the less labile P pools, NaOH-P inorganic was depleted in the rhizosphere of faba bean while NaOH-P organic and residual P, were most strongly depleted in the rhizosphere of white lupin. The results suggest that even in presence of labile P, less labile P pools may be depleted in the rhizosphere of some legumes.

Keywords

Grain legumes, P availability, P fractionation, Species variation

Introduction

Of the applied P, only 20-30% are taken up by the crop in the first year, the rest is mainly fixed or precipitated into less labile P forms (Bünemann et al. 2006; Richardson et al. 2001; Vu et al. 2008). The amount of P fixed or precipitated in the soil depends on the soil pH and existing minerals. Due to the low P fertiliser efficiency, farmers often apply P fertilisers in excess of plant requirement to sustain crop production; this practice has resulted in a build-up of residual P and non-labile inorganic P in the soil (Vu et al. 2008). In Australia, it has been estimated that this soil P bank is equivalent to $5-10 billion worth of P fertilisers (Stevens et al. 1997). Plant species, particularly legumes, have various strategies to mobilise P from less labile P pools. Previous studies showed legumes can mobilise more P from less labile P than cereals (Kamh et al. 1999; Nuruzzaman et al. 2005a, b).

The ability of legumes to mobilise P from less labile soil P pools is thought to be due to release of organic acid anions such as malate and citrate and acid phosphatases from roots (Lambers et al. 1998; Nuruzzaman et al. 2006). Organic acid anions can mobilise soil P pools by reducing the number of binding sites for P fixation via chelation of Fe and Al (Gerke 1992) and by replacing P from adsorption sites (Nziguheba et al. 2000). The release of acid phosphatase (Tarafdar and Claassen 2003) and phytase (Richardson 2001) plays an important role in mobilisation of organic P. Rhizosphere acidification resulting from proton release during N_2 fixation (Hinsinger et al. 2003; Tang et al. 1998) is another process which enhances P availability in alkaline soils because the solubility of Ca phosphates increases with decreasing pH. This ability to mobilise P by legumes may also explain the increase in growth and P uptake of wheat following legumes in rotation (Nuruzzaman et al. 2005b).

In most studies on mobilization of P, the plants were grown in soil with low P availability because the P mobilization capacity, e.g. release of organic acid anions, is enhanced under P

deficiency (Neumann et al. 1999). However, growers often apply P fertiliser to reduce the risk of P deficiency during legume growth and to improve nodulation and N_2 fixation. In white lupin, high P availability reduces the number of cluster roots and the release of carboxylates (Neumann et al. 1999). It is therefore unclear if P mobilisation also occurs at high P supply. We addressed this knowledge gap by comparing five legume species in their response to P addition and ability to access various P pools in an alkaline soil with low P availability to which P was added. We hypothesised that at high P availability, legumes will deplete mainly the labile P pools and will not deplete less labile P pools. The characterisation of the changes in soil P pools caused by legumes is important for a better understanding of species variation in mobilising P pools which could provide information for farmers when selecting legume species for either crop rotation or intercropping.

Materials and Methods

Soil

A non-calcareous loamy sand soil under natural vegetation (0-10 cm) was collected in Monarto, (latitude 35° 05′ S, longitude 139° 04′ E and elevation 212 m), South Australia. The soil was air-dried and passed through a 2 mm sieve. The properties of the soil are as follows: pH (1:5 soil/water) 8.8, clay 7.5%, sand 82.5%, silt 10%, organic C 0.7%, total N 0.09%, total P 146.4 mg kg^{-1}, resin P 4.5 mg kg^{-1}, NO_3^- 31.2 mg kg^{-1}, NH_4^+ 28.1 mg kg^{-1}, bulk density 1.6 g cm^{-3}, maximum water holding capacity 160 g kg^{-1} and P buffering index 49. Soil pH was measured after shaking soil in a 1:5 soil/water ratio for 1 h. Resin P was determined using anion-exchange resin membrane (BDH #55164) converted to bicarbonate form (Kouno et al. 1995). Total P was determined by wet digestion of a mixture nitric and perchloric acid (6:1) (Kuo 1996) and the P concentration was determined colorimetrically using the molybdenum blue method (Murphy and Riley 1962). Nitrogen in soil was extracted using 2 M KCl as described by Keeney and Nelson (1982). Nitrate was measured by cadmium reduction method (Henrikson and Selmer-Olsen 1970), while ammonium was measured by the

nitroprusside/dichloro-S-triazine modification of the Bertelot indophenol reaction (Searle 1984). Water holding capacity was measured by a gravimetric method. Phosphorus buffering index (PBI) was measured according to Rayment and Lyons (2010). Soil P pools were determined using a modified version of the sequential extraction from Tiessen and Moir (1993) (see below). To determine the effect of fertiliser addition on the initial size of the P pools, the soils were analysed either without fertiliser addition or one day after the addition of the fertiliser prior to the sowing.

Plant growth conditions

A preliminary experiment was conducted with faba bean over six weeks to determine the amount of added P required for maximal growth, with P addition ranging from 5 to 200 mg P kg^{-1} as KH_2PO_4. Maximal growth was reached at 80 mg P kg^{-1}. There was no further growth increase at higher P addition rates. Therefore 80 mg P $kg^{-1}$was used in the experiment described here.

The experiment was carried out in a glasshouse between October and January (spring to summer). The temperature in the glasshouse during the experiment ranged from 15 to 30 ºC. The soil was carefully mixed with 80 mg P kg^{-1} as KH_2PO_4 and placed in 1.9 L pots lined with a polyethylene bag. Four pre-germinated seeds of faba bean (*Vicia faba* L. cv. Fiesta) (FB), chickpea (*Cicer arietinum* L. cv. Kabuli) (CP), narrow-leafed lupin (*Lupinus angustifolius* L. cv. Wonga) (NL) yellow lupin (*Lupinus luteus* L. cv. Wodjil) (YL) and white lupin (*Lupinus albus* L. cv. Luxor) (WL) were sown per pot. These grain legumes were selected based on previous studies (Nuruzzaman et al. 2005a, b; Tang et al. 1995) for their differential response to soil pH and mechanisms in accessing soil P. Further, these legumes are commonly used in Australian farming systems. Faba bean and chickpea are the most common legumes grown in alkaline soils in Southern Australia. The model plant for P mobilisation white lupin, is known for the cluster root formation and carboxylate release under P deficiency (Neumann and Römheld 2002). Yellow lupin and narrow-leafed lupin are

commonly grown in acidic soil in Western Australia. Five days after sowing, each legume was inoculated with an appropriate rhizobium strain: strain WU425 (group G) for NL, YL and WL; strain WSM1455 (group F) for faba bean; rhizobium strain CC1192 (group N) for chickpea. Two weeks after sowing, the plants were thinned to two plants per pot. The soil was maintained at 70% field capacity by adding reverse osmosis (RO) water by weight throughout the experiment. Basal nutrients, except N and P were supplied once a week as described in Nuruzzaman et al. (2005b). The composition of the nutrient solution was (μM): $CaCl_2$, 150; K_2SO_4, 100; $MgSO_4$, 54; H_3BO_3, 2.4; $MnSO_4$, 0.24; $ZnSO_4$, 0.1; Na_2MoO_4, 0.03; $CuSO_4$, 0.02; $CoCl_2.6H_2O$, 0.001. The nutrient solution (pH 5.4) was added at 100 mL per pot and week initially; after four weeks the volume was increased to 200 mL per pot and week until the end of the experiment. Unplanted pots serving as controls and representing the bulk soil received the same amount of nutrients and also maintained at 70% field capacity. The pots were arranged in a complete randomised design. Plants were harvested at flowering and maturity, the harvesting times varied slightly with legume species, but were at approximately 45 days after sowing (DAS) for flowering and approximately 70 DAS for maturity. These two stages were chosen to study because of the differences in P demand. Until flowering, the demand for P will be high and plant P uptake is expected to be maximal at maturity. There were four replicate pots per legume species and the unplanted control at each harvest.

Plant and soil analyses

At harvest, the shoots (including seeds at maturity) were cut at the soil surface, rinsed in reverse osmosis (RO) water and then oven-dried at 80ºC for two days. The roots were gently removed from the pots and soil loosely adhering to the roots was shaken off, leaving the rhizosphere soil (strongly adhering soil) which was collected by gentle brushing, and stored at -20°C for analysis of soil total P and P fractionation. There was approximately 10 - 15 g of rhizosphere soil per pot. The unplanted pots were used to collect bulk soil. Plant materials

were ground separately and digested in nitric and perchloric acid mixture (6:1) for five hours, total P was measured using the vanado-molybdate method (Hanson 1950).

For determination of soil P pools, sequential fractionation was carried out using the method described by Tiessen and Moir (1993) with slight modifications. The procedure removes the most labile P followed by less labile P forms by using mild to stronger extractants sequentially (Tiessen and Moir 1993). Briefly, 1 g of sieved rhizosphere and bulk soil (<0.5 mm) was weighed and extracted sequentially by shaking overnight (16 h) with 30 ml of the following solutions: 1) water (resin P); 2) 0.5 M $NaHCO_3$; 3) 0.1 M NaOH; 4) 1 M HCl and 5) 0.1 M NaOH. Another set of tubes with the same soil samples were prepared to measure microbial P using the resin method. Anion-exchange resin membrane (BDH #55164) was added in the tubes with 30 mL of water and 1 mL hexanol as the fumigant. Phosphorus in the resin for both sets (fumigated and non-fumigated) was then eluted with 0.1 NaCl/HCl and the difference of P concentration was considered to be microbial P. The inorganic P (Pi) concentration in all extracts was determined using the molybdenum blue method (Murphy and Riley 1962). Total P in the 0.5 M $NaHCO_3$ and 0.1 M NaOH fractions was determined by digestion of the extract with sulphuric and perchloric acid (6:1) for 10 h (Kuo 1996). Organic P (Po) in these fractions was calculated by the difference of total P and inorganic P fractions (Pi). Organic P in the HCl fraction was not determined as preliminary tests showed that the amount of organic P in this fraction is very low, which is in agreement with findings of Tiessen and Moir (1993). Double extraction with 0.1 M NaOH was carried out as previous studies showed that there were significant amounts of NaOH extractable P after the extraction with 1 M HCl (Bünemann, personal communication). Residual P in the remaining soil and soil total P of un-fractionated soil were determined by digesting the samples in nitric and perchloric acid mixture (6:1) for 4 h. Resin P and bicarbonate-extractable ($NaHCO_3$) Pi and Po are considered as labile pools (Tiessen and Moir 1993; Linquist et al. 1997). Sodium

hydroxide-extractable (NaOH) Pi and Po, acid-extractable (HCl) Pi and residual P were defined as less labile pools (Hedley et al. 1982; Tiessen and Moir 1993).

The relative contribution of each P pools to P uptake was calculated as:

relative contribution (%) = (P concentration of pool at flowering (mg kg^{-1}) - P concentration of pool at maturity (mg kg^{-1}))/ total of depleted pools (mg kg^{-1}) x 100.

Statistical analysis

Statistical analysis was performed using GenStat® for Windows 10.0 (VSN Int. Ltd, UK). The differences among the treatments were established by the analysis of variance (ANOVA), post-hoc analyses were performed using Tukey multiple comparison test at $P \leq 0.05$.

Results

Growth and P uptake

The weight per seed (g) at sowing was: faba bean (0.66) > chickpea (0.33) > white lupin (0.32) > yellow lupin and narrow-leafed lupin (0.15). Whereas faba bean, chickpea and white lupin grew well, narrow-leafed lupin and yellow lupin grew poorly. Nodulation was observed in all legumes but cluster roots were only found in white lupin. At flowering, shoot biomass ranged from 3.1 to 7.4 g pot^{-1} and root biomass from 1.0 to 3.6 g pot^{-1} (Table 1). White lupin had the highest shoot and root biomass with 7.4 and 3.6 g pot^{-1}. Biomass increased from flowering to maturity in all legumes. On average, shoot biomass (plus seeds) at maturity was increased by 12.5 to 26.1 g pot^{-1} and root biomass increased by 2.6 to 9.9 g pot^{-1}. Shoot and root biomass were highest in faba bean while white lupin had the lowest shoot biomass and yellow lupin had the lowest root biomass. Faba bean and chickpea had a fibrous root system whereas that of the lupins was coarser.

The P concentration in plants significantly decreased from flowering to maturity in all legumes except white lupin (Table 1). At flowering, faba bean had the highest and white lupin

had the lowest shoot P concentration. The root P concentration was highest in chickpea and lowest in white lupin. At maturity, shoot P concentration was greatest in narrow-leafed lupin and root P concentration was greatest in chickpea, while yellow lupin had the lowest shoot and root P concentrations.

Plant P uptake increased 1.5 to 4 fold from flowering to maturity with the greatest increase in chickpea and the lowest in white lupin (Table 1). P uptake was highest in faba bean both at flowering and maturity, whereas it was lowest in yellow lupin at flowering and in white lupin at maturity.

Soil pH and size of soil P pools

At flowering and maturity, the pH of the bulk soil from the unplanted pots and the rhizosphere of all legumes was significantly lower than in the initial soil (pH 8.8). The pH decrease was the greatest in the bulk soil and the rhizosphere soil of faba bean, being 0.9 units lower than the initial soil (data not shown). The pH decrease was smallest in the rhizosphere of white lupin (pH 8.5).

Compared to the original soil, the addition of soluble P resulted in an increase of the size of all P pools, particularly the inorganic pools (Figure 1). Resin P was increased 11 fold, followed by microbial P (5 fold), HCl-Pi (2.4 fold), $NaHCO_3$-Pi (2.4 fold), NaOH-Po, NaOH-Pi, $NaHCO_3$-Po and residual P by 85, 73, 19 and 11%; respectively.

Generally, the changes in P pools in the rhizosphere compared to the bulk soil were more pronounced at maturity than at flowering, therefore only the P pools at maturity are described here (Figure 2a-e). Irrespective of the legume species, the sum of inorganic P pools in the rhizosphere soil was greater than the sum of organic P pools, with resin P being the largest, followed by HCl-, NaOH- and $NaHCO_3$-Pi. Residual P ranged from 40-47% of total P. The organic P pool ranged from 10-19% of total P, with NaOH-Po being larger than $NaHCO_3$-Po.

The concentration of microbial P was low and similar in all treatments including the bulk soil. Compared to the bulk soil, the inorganic labile pool resin P was significantly lower in the rhizosphere of all legumes, with the strongest decrease in faba bean (by 54%) and the smallest in yellow lupin (by 34%) (Figure 2a). Another inorganic labile pool, $NaHCO_3$-Pi, was significantly lower than in the bulk soil in the rhizosphere of faba bean (by 39%) followed by chickpea (by 31%). Conversely, the organic labile pool $NaHCO_3$-Po was significantly higher in the rhizosphere of yellow lupin and narrow-leafed lupin compared to the bulk soil and the other legumes (Figure 2b). The concentration of the less labile pool NaOH-Pi did not differ between rhizosphere and bulk soil except in the rhizosphere of faba bean, where it was decreased by 21%. Compared to the bulk soil, the concentration of NaOH-Po was significantly higher in the rhizosphere of faba bean and chickpea; however it was lower in the rhizosphere of white lupin (by 37%) where it was also significantly lower than in the other legumes (Figure 2c). There were no significant differences in the HCl-Pi pool between the rhizosphere and the bulk soil except in white lupin, which had a significantly higher HCl-Pi concentration than the other legumes and the bulk soil (Figure 2d). Another less labile pool, residual P was 25% lower in the rhizosphere of white lupin than in the bulk soil and the other legumes. Soil total P, which was determined separately, was significantly lower in the rhizosphere than in the bulk soil except in narrow-leafed lupin, but did not differ among legumes (Figure 2e).

Relative depletion in soil P pools from flowering to maturity

Table 2 shows the contribution of the different P pools to the total decrease in P concentration in the rhizosphere from flowering to maturity. In most legumes, resin P contributed most to the depletion (36-57%), followed by $NaHCO_3$-Pi and HCl-Pi and small contributions from organic P pools. In contrast, in the rhizosphere of white lupin the contribution of less labile pools was greatest: 43% from residual P and 20% of NaOH-Po, followed by the labile pools (resin P, $NaHCO_3$-Po and $NaHCO_3$-Pi) and the contribution of NaOH-Pi was lowest.

Discussion

This study showed that even though addition of soluble P resulted in an increase in the size of the labile P pools, legumes differed in their ability to deplete this labile P and some of the less labile pools. The first part of our hypothesis that at high P availability, legumes will deplete mainly the labile P pools holds true for most legumes. However, the second part of the hypothesis (and will not deplete less labile P pools) has to be rejected as some legumes depleted less labile P pools (NaOH-Pi, NaOH-Po and residual P) and in white lupin the contribution of the less labile P pools to the decrease in P in the rhizosphere was greater than that of the labile P pools.

Faba bean had the greatest biomass compared to the other legumes in this experiment which is in agreement with the study by Nuruzzaman et al. (2005b) in an acid soil (pH_{CaCl2} 5.3) and Rose et al. (2010) in both acid and alkaline soil. Yellow lupin and narrow-leafed lupin showed stunted growth from the beginning of the experiment, due to the high pH of the soil (pH 8.8) as these species are well adapted to acid soil (French et al. 2001). Narrow-leafed lupin is known to be sensitive to high pH (Tang et al. 1995; Tang and Robson 1993). Poor growth and severe chlorosis of different yellow lupin genotypes in alkaline soils was also observed by Tang et al. (1995).

All legumes significantly depleted resin P beyond the decrease in the control soil (Figure 2a), suggesting that this labile pool served as the principal source of plant available P. Vu et al. (2008) also found a depletion of labile P pools (water- and $NaHCO_3$-Pi) at high P supply; but in contrast to the present study, they also found that chickpea decreased the acid-extractable fractions (HCl-Pi and H_2SO_4-Pi). A similar result was reported by Rose et al. (2010) where chickpea depleted HCl-Pi particularly in an acidic clay loam soil (pH 6.1). In the present study, however, HCl-Pi which represents Ca-bound P, was not significantly lower in the rhizosphere of the legumes compared to bulk soil, suggesting that this fraction was not accessible in this particular soil. The contrasting results may be explained by the duration of

the experiment: in the study by Vu et al. (2008) chickpea was grown twice (120 days) and by the lower pH (7.4) of their soil. Several other studies with neutral and acidic soils also reported a decrease in the acid-extractable pool by various crops particularly legumes (Kamh et al. 1999; Li et al. 2008; Wang et al. 2008). The lack of depletion of HCl-Pi in our study may thus be due to the high pH of the soil and the fact that although the pH was decreased in the rhizosphere, it remained above pH 7.9.

Faba bean and chickpea strongly depleted the labile P pools, resin P and $NaHCO_3$-Pi and these two pools contributed most to the decrease in P concentration in the soil from flowering to maturity. Bicarbonate-extractable P includes labile P compounds such as P adsorbed to free lime, sorbed onto iron and aluminium oxides and clay minerals and slightly soluble calcium phosphate (Schoenau and O'Halloran 2008). The strong depletion of $NaHCO_3$-Pi by faba bean and chickpea suggests that these species are able to access labile P to a greater extent than the other legumes (Figure 2b). Additionally, the less labile P pool NaOH-Pi was depleted in the rhizosphere of faba bean. The NaOH-Pi pool represents inorganic P strongly associated with iron and aluminium on soil surfaces (Hedley et al. 1982; Tiessen and Moir 1993). Its depletion in the rhizosphere suggests that faba bean released carboxylates that released by P by anion exchange or solubilisation of Fe and Al (Gerke 1992). The strong depletion of these pools explains the strong increase in P uptake in these two legumes from flowering to maturity. The ability to take up large amounts of labile P is likely to be due to the high root biomass of faba bean and chickpea compared to the other legumes. Nuruzzaman et al. (2005a) attributed the highest P uptake of faba bean to its larger root dry mass compared to field pea, white lupin and wheat. In the study by Vu et al. (2008), the $NaHCO_3$-Po was strongly decreased in the rhizosphere of chickpea. This is in contrast to the present study however where $NaHCO_3$-Po was higher in the rhizosphere of chickpea than in the bulk soil. The concentration of NaOH-Po was also significantly higher in the rhizosphere of faba bean and chickpea compared to the bulk soil. The increase in the organic P pools could be due to the

transformation of inorganic into organic P forms by soil microorganisms and this transformation may be enhanced in the rhizosphere due to the increased carbon input compared to the bulk soil (Grayston et al. 1997). Further, dead roots may have contributed to the organic P pools.

Despite the addition of soluble P, white lupin produced many cluster roots which in agreement with a study by Pearse et al. (2006). It has been suggested that cluster root formation is a function of the internal Pi requirement of the plant rather than soil P (Li and Liang 2005; Marschner 1995). In the present study, the shoot P concentration of white lupin at flowering was below the critical shoot P concentration for this plant species (Reuter and Robinson 1997), however, the shoot P concentration at maturity was adequate whereas it was deficient in the other legumes at both sampling times. It appears that the formation of cluster roots in white lupin was stimulated by P deficiency at the early stages of growth, which then enhanced the acquisition of P from soil to support the P demand at later stages. The decrease in the less labile P pools, NaOH-Po and residual P in the rhizosphere of white lupin and their high contribution to the total reduction in P pools is probably due to the release of phosphatase and carboxylates such as citrate and malate by the cluster roots (Nuruzzaman et al. 2005a, b; Veneklaas et al. 2003). Soil organic P must be hydrolysed by phosphatase in order to become available to plants (Richardson et al. 2001). George et al. (2006) showed the depletion of the NaOH-Po pool was positively correlated with the activity of acid phosphatase in the rhizosphere of tithonia and transgenic clover. The depletion of less labile pools by white lupin is in agreement with a study by Kamh et al. (1999) in a soil with low P availability but our study shows that this occurs even if the concentration of labile P is quite high. Interestingly, the depletion of the less labile P pools occurred although the root biomass of white lupin was high. However, the high root biomass by white lupin may be mainly due to the cluster roots and therefore does not necessarily indicate a high root length. Upon visual observation at harvest, the root systems of all lupins appeared to be coarser than those of faba

bean and chickpea. Despite the depletion of the less labile P pools, NaOH-Po and residual P in the rhizosphere, P uptake from flowering to maturity by white lupin was quite low, suggesting that P mobilised from these P pools was not taken up by the plant. Indeed, some of the P mobilised from these pools may have been converted into HCl-Pi as this pool was significantly increased compared to the other legumes (Figure 2d).

The lack of depletion in most soil P pools by yellow lupin and narrow-leafed lupin (except in resin P) is most likely due to the poor growth of these legumes.

Conclusions

Even in the presence of relatively high concentrations of labile P, the legumes had a differential capacity to grow and take up P and change soil P pools. Most legumes depleted labile P pools, but faba bean and white lupin were able to deplete P in less labile pools (NaOH-Pi, Po and residual P). However, the depletion of some P pools was associated with increases in others, particularly the organic P pools which suggests conversion of P among P pools. The contribution of the various P pools to P uptake by the plant could not be quantified in the present study, but this could be done by adding labelled P to the soil and incubating the soil for some time to allow transformation in the various P pools. Further, in situ measurement of carboxylates and phosphatase activity in the rhizosphere would allow determination of the mechanisms of P mobilisation (Li et al. 2010).

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Table 1: Plant biomass, P concentration and P uptake of five legumes at flowering and maturity. Data represent means (n=4), within columns, means followed by the same letter are not significantly different (P≤ 0.05) by Tukey's multiple comparison tests.

Legume species	Biomass (g pot^{-1})				P concentration (mg g^{-1})				P uptake (mg P pot^{-1})	
	shoot		root		shoot		root			
	flowering	maturity	flowering	maturity	flowering	maturity	flowering	maturity	flowering	maturity
Faba bean	5.5 abc	26.1 c	3.1 bc	9.9 e	5.8 d	2.3 bc	3.5 c	1.6 ab	36.5 c	85.1 b
Chickpea	3.1 a	23.1 c	1.6 ab	8.4 e	4.3 c	2.1 b	5.4 d	2.6 d	19.7 ab	78.0 b
Narrow-leafed lupin	3.5 ab	14.4 b	1.6 ab	3.8 bc	4.3 c	2.7 cd	3.4 b	2.0 bc	19.8 ab	45.8 a
Yellow lupin	3.7 ab	12.6 ab	1.0 a	2.6 abc	3.3 b	1.0 a	3.3 b	1.3 a	16.7 a	45.5 a
White lupin	7.4 cd	12.5 ab	3.6 c	6.2 d	2.1 a	2.1 b	2.3 a	1.6 bc	25.2 b	38.1 a

Table 2: Relative contribution of the P pools as percentage of the total reduction (%) in the rhizosphere soil of five legumes from flowering to maturity.

Legume species	**Microbial P**	**Resin P**	**$NaHCO_3$-Pi**	**$NaHCO_3$-Po**	**NaOH-Pi**	**NaOH-Po**	**HCl-Pi**	**Residual P**
Control		38.0	9.6	3.1		16.6	19.3	13.5
Faba bean	5.8	36.4	17.7	3.6		16.9	19.5	
Chickpea	2.9	57.1	13.3			16.0	10.8	
Narrow-leafed lupin	10.2	45.8				44.0		
Yellow lupin	2.1	43.0	3.4			43.5	8.1	
White lupin		15.0	5.8	11.8	4.7	20.2		42.5

List of figures

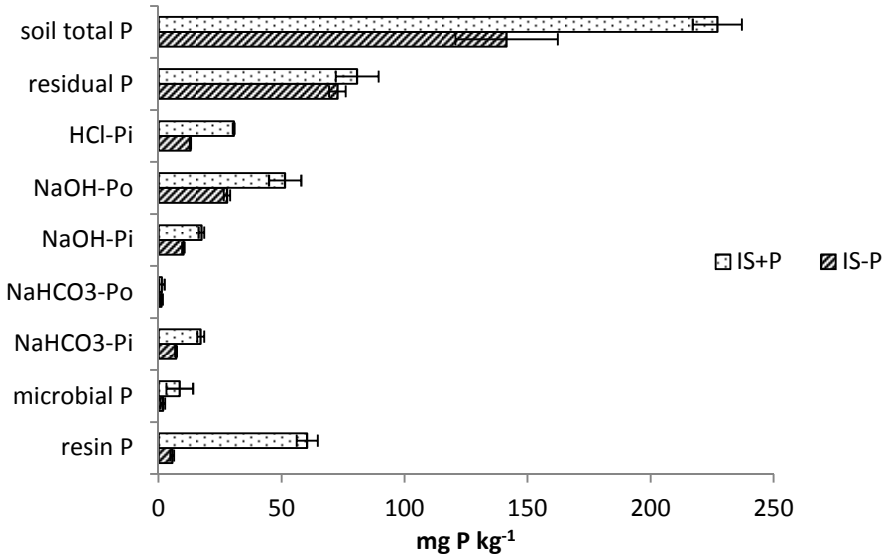
soil total P
residual P
HCl-Pi
NaOH-Po
NaOH-Pi
NaHCO3-Po
NaHCO3-Pi
microbial P
resin P
IS+P
IS-P
0
50
100
150
200
250
mg P kg-1

Figure 1

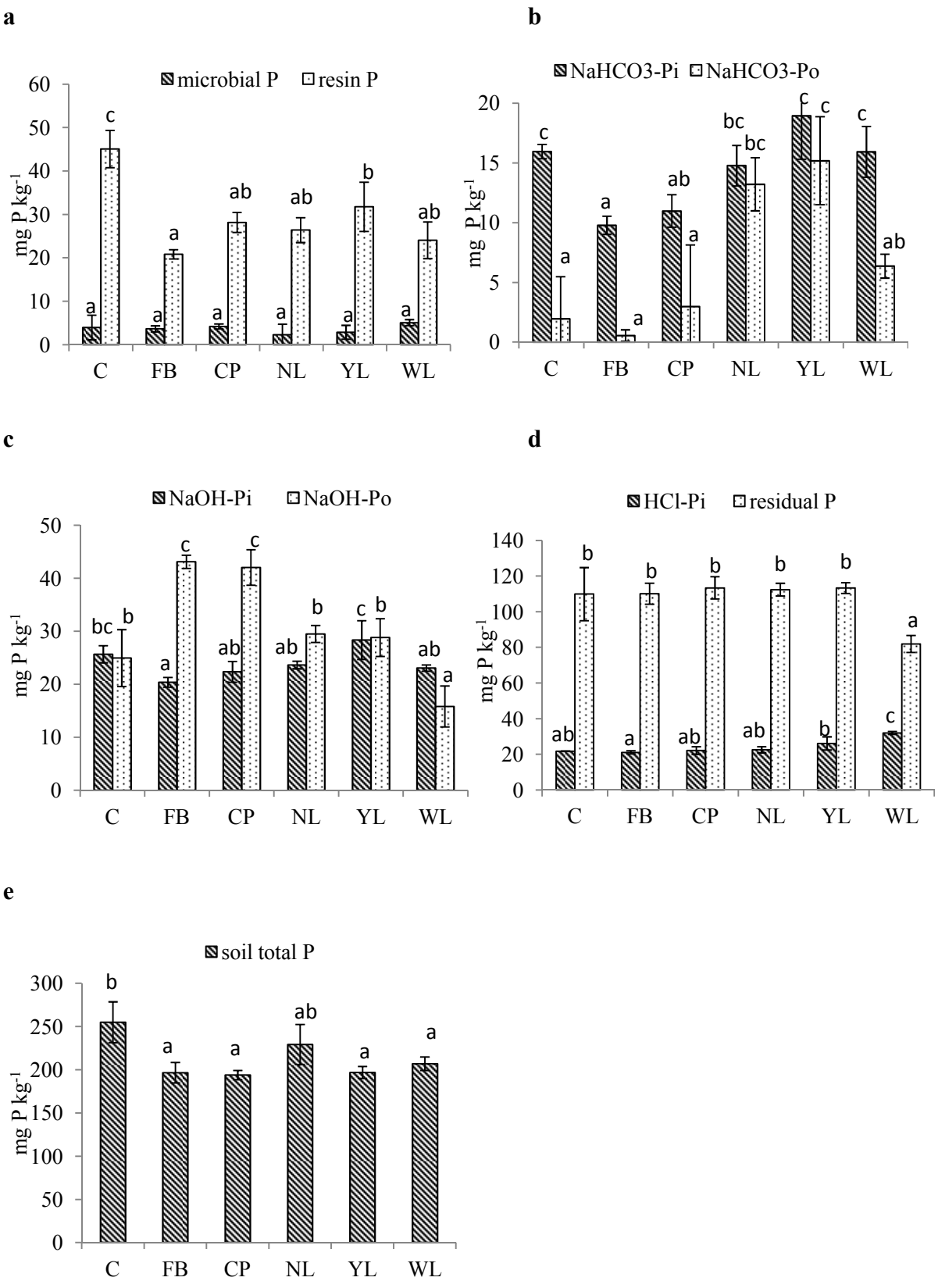
a
microbial P
resin P
mg P kg-1
C
FB
CP
NL
YL
WL
b
NaHCO3-Pi
NaHCO3-Po
c
NaOH-Pi
NaOH-Po
d
HCl-Pi
residual P
e
soil total P

Figure 2

Chapter 3

Grain legume pre-crops and their residues affect growth, P uptake and the size of P pools in the rhizosphere of the following wheat

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Biology and Fertility of Soils

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STATEMENT OF AUTHORSHIP

Grain legume pre-crops and their residues affect growth, P uptake and the size of P pools in the rhizosphere of the following wheat

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Mat Hassan, H (Candidate)

Performed analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author.

I hereby certify that the statement of contribution is accurate.

Signed Date

Marschner, P

Supervised development of work, data interpretation and manuscript evaluation.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date

McNeill, Ann

Manuscript evaluations

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Signed Date

Tang, Caixian

Manuscript evaluations

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Grain legume pre-crops and their residues affect growth, P uptake and the size of P pools in the rhizosphere of the following wheat

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Abstract

Legumes have been shown to increase P uptake of the following cereal, but the underlying mechanisms are unclear. The aim of this study was to compare the effect of legume pre-crops and their residues on the growth, P uptake and the size of soil P pools in the rhizosphere of the following wheat. Three grain legumes (faba bean, chickpea and white lupin) were grown until maturity in a loamy sand soil with low P availability to which 80 mg P kg^{-1} was supplied. This pre-crop soil then was amended with legume residues or left un-amended and planted with wheat. Growth, P uptake and the concentrations of P pools in the rhizosphere of the following wheat were measured 6 weeks after sowing. In a separate experiment, residue decomposition was measured over 42 days by determining soil CO_2 release as well as available N and P. Decomposition rates were highest for chickpea residues and lowest for wheat residues. The P release was greatest from white lupin residues and N release greatest from faba bean residues while wheat residues resulted in net N and P immobilisation. The growth of the following wheat was greater in legume pre-crop soil without residue than in soils with residue addition while the reverse was true for plant P concentration. Among the

legumes, faba bean had the strongest effect on growth, P uptake and concentrations of the rhizosphere P pools of the following wheat. Regardless of the pre-crop and residue treatment, wheat depleted the less labile pools residual P as well as NaOH-Pi and Po, with a stronger depletion of the organic pool. We conclude that although P in the added residues may become available during decomposition, the presence of the residues in the soil had a negative effect on the growth of the following wheat. Further, pre-crops or their residues had little effect on the size of P pools in the rhizosphere of wheat.

Keywords

Decomposition, Grain legumes, P fractionation, P pools, Rhizosphere, Rotation

Introduction

Grain and forage legumes are currently grown on 180 million ha worldwide and their extent is expected to increase as the demand for legume production for dietary protein increases (Graham and Vance 2003). Several studies showed positive effects of legumes on cereals either in rotation or intercropping (Richardson 2001; Kamh et al. 2002; Nuruzzaman et al. 2005a, b) which cannot be solely explained by N input through N_2 fixation. Some legumes have a greater ability to mobilise P from less labile P forms than cereals (Kamh et al. 1999; Nuruzzaman et al. 2005a, b). It has been suggested that mobilisation of soil P by exudation of organic acid anions from legume roots may enhance the growth of cereals following legumes (Kamh et al. 1999). This suggestion however has been challenged due to rapid decomposition of organic acid anions in soil (Jones et al. 2003; Nuruzzaman et al. 2005a, b). Therefore, it seems more likely that the beneficial effect of these root exudates on P availability plays a role in intercropping where the roots of the legume and cereal intermingle. Another explanation for the increased cereal growth and P uptake after legumes is P release during decomposition of legume residues (Horst et al. 2001; Kamh et al. 2002; Nuruzzaman et al. 2005a, b).

Increased P availability in soil amended with crop residues with high P concentration can be attributed to P released during decomposition (Bünemann et al. 2008) which may enhance the growth and P uptake of following crops (Nziguheba et al. 2000). The C/P ratio of the added residues is an important indicator for the ratio of P mineralisation to immobilisation. Crop residues with C/P ratios less than 300 are likely to induce net P mineralisation while net immobilisation is likely at C/P >300 (Cheshire and Chapman 1996; Brady and Weil 2002). However, other residue properties such as total P, water-soluble P and C, and N/P ratio also influence the ratio of P immobilisation to P mineralisation (Nziguheba et al. 2000). Due to the greater capacity of legumes to utilise soil P, it is expected that legume residues contain more P and have lower C/P ratios than cereals which may favour net P mineralisation, thereby increasing growth and P uptake of the following wheat. Residues also increase soil P availability by stimulation of the microbial production of organic acid anions and phenolic compounds that can lead to mobilisation of adsorbed P by competing for P adsorption sites (Hu et al. 2005). Further, residue addition may change the soil pH (Xu et al. 2006), e.g., application of pearl millet residues increased the soil pH and base saturation, thus decreasing exchangeable Al and thereby decreasing P sorption (Hafner et al. 1993).

To maximise the beneficial effects of legumes in crop rotation, it is important to understand which legumes may increase the growth and P uptake of the following wheat and by which mechanisms they do so. However there are no published data on the effects of pre-crop legumes and their residues on the growth, P uptake and P pools in the rhizosphere of the following wheat. In a previous study, we showed that grain legumes have differential effects on the concentrations of various P pools (Mat Hassan et al. 2011). The soil and the residues from that study were used in the experiment described here. The aims of this study were to (i) compare decomposition rate and changes in N and P availability during decomposition of wheat and grain legume residues, and (ii) assess the effects of pre-crop legumes with or

without residue addition on growth, P uptake and concentrations of P pools in the rhizosphere of the following wheat.

Materials and methods

Soil and plant materials

The soil (0 – 10 cm) used for the experiments was collected in Monarto, South Australia (latitude 35° 05′ S, longitude 139° 04′ E, elevation 212 m). The soil was air-dried and passed through a 2-mm sieve. It had the following properties: clay 7.5%; sand 82.5 % and silt 10 % (loamy sand); pH (H_2O) 8.8; total organic C 0.7 %; total N 0.09%; total P 146.4 mg kg^{-1}; resin extractable P 4.5 mg kg^{-1} and maximum water-holding capacity (WHC) 160 g kg^{-1}. The high pH in this non-calcareous soil is due to the presence of sodium. Three grain legumes: faba bean (*Vicia faba* L. cv. Fiesta), chickpea (*Cicer arietinum* L. cv. Kabuli) and white lupin (*Lupinus albus* L. cv. Luxor) were grown to maturity (Mat Hassan et al. 2011). The experiment also included an unplanted control soil with four replications for each treatment (n=4). The soil from each legume and from the unplanted control was then air-dried and stored for 3 months before being used for the experiments described here in which the soil and the legume residues were used for an incubation study and a glasshouse experiment with wheat. Mature wheat residue was obtained from a field experiment. The residues (vegetative parts of the shoots and roots) were oven-dried at 70°C for 48 h, ground and sieved to 0.25-2 mm. Some properties of the residues are shown in Table 1.

Experimental design

Incubation study

Previously unplanted Monarto soil was pre-incubated at 70% WHC for two weeks to avoid a flush of microbial activity after rewetting of dry soil (Fierer et al. 2003). After this pre-incubation, residues were thoroughly mixed with the soil at a rate of 20 g kg^{-1}. This rate was used to assess the maximal impact of the residues on nutrient availability. The experiment

consisted of 12 treatments (4 replicates per sampling date): faba bean shoots (Fs), roots (Fr), and mixed shoot and roots (1:1, Fm); chickpea shoots (Ps), roots (Pr), and mixed shoot and roots (Pm); white lupin shoots (Ls), roots (Lr), and mixed shoot and roots (Lm); and wheat shoots (Ws), roots (Wr), and mixed shoot and root (Wm). Soil without residues served as control and was mixed in the same way as the soils with residue addition. Twenty five grams of soil was incubated in PVC cores (5 cm height, 3.7 cm diameter) with a nylon mesh base (0.75 μm) and adjusted to a bulk density of 1.6 g cm^{-3}. These PVC cores were placed into 1 L glass jars with gas-tight lids equipped with septa for measurement of headspace CO_2 concentration. Tubes with reverse osmosis water (RO) were placed inside the jars to maintain a humid atmosphere and to minimise soil moisture loss during the incubation. The jars were incubated at 22-25°C in the dark. Autoclaved RO water was added to maintain the soil water content at 70% WHC by weighing the cores every 3 days. The remaining cores were placed in large plastic containers with loosely fitting lids and incubated under the same conditions as the glass jars, their water content was also maintained by regular watering to weight. Respiration rate of the samples in the jars was measured daily until day 8 and then every 3 days until day 42 with a Servomex 1450 Infra-red gas analyser (Servomex group, Crowborough, England). After each measurement, the jars were opened to equilibrate the CO_2 concentration in the jars to ambient concentrations and then resealed. The CO_2 evolved from each sample was calculated as the difference between the initial concentration (immediately after resealing of the jars) and that at the end of the measuring interval. At the first destructive sampling on day 21, the cores were removed from the jars for subsequent analyses and the cores previously incubated in the large plastic containers were placed in the jars for respiration measurement until the next sampling on day 42. The samples from day 0, 21 and 42 were analysed for resin (available) P and available N.

Resin (available) P was determined using anion-exchange resin membrane (BDH #55164) converted to bicarbonate form according to Kouno (1995), and the P concentration was

determined colorimetrically using the molybdenum blue method (Murphy and Riley 1962). Soil available N was extracted using 2 M KCl as described by Keeney and Nelson (1982). Nitrate was measured by the cadmium reduction method (Henrikson and Selmer-Olsen 1970), and ammonium was measured by the nitroprusside/dichloro-S-triazine modification of the Berthelot indophenol reaction (Searle 1984). The inorganic N concentration reported below is the sum of ammonium and nitrate concentrations.

Wheat growth at different rates of faba bean residues

To assess the impact of residue addition rate on wheat growth, wheat was grown in Monarto soil with faba bean residues at 0-30 g kg^{-1} soil. Total biomass (shoot plus root) was determined after 6 weeks.

Wheat growth in pre-crop soil with and without residues

Wheat (*Triticum aestivum* L. cv Krichauff) was grown in previously unplanted control soil, pre-crop soils without residues (soil in which faba bean, chickpea or white lupin had previously grown) and pre-crop soils with residue addition. Plant residues (vegetative parts of the shoots and roots) were added at 8 g kg^{-1} and thoroughly mixed before planting of the wheat. A different rate compared to the incubation experiment was used as the experiment with different faba bean addition rates showed that wheat growth was reduced by 50% or more at addition rates of $\geq$ 15 g kg^{-1} (Figure 1). The treatments, each with 3 replications (n=3) were: wheat grown in unplanted control soil (W-C); faba bean pre-crop soil without residues (W-F), with shoot residues (W-Fs), with root residues (W-Fr); chickpea pre-crop soil without residues (W-P), with shoot residues (W-Ps), with root residues (W-Pr); and white lupin pre-crop soil without residues (W-L), with shoot residues (W-Ls), with root residues (W-Lr). Four pre-germinated wheat seeds were sown 2 cm deep in each non-draining pot (300 g) and thinned to 2 plants per pot seven days after sowing. Nitrogen was supplied weekly as ammonium nitrate at 2.4 mg N kg^{-1}. Nutrient solution (except P) with the following

composition (μM): $CaCl_2$, 150; K_2SO_4, 100; $MgSO_4$, 54; H_3BO_3, 2.4; $MnSO_4$, 0.24; $ZnSO_4$, 0.1; Na_2MoO_4, 0.03; $CuSO_4$, 0.02; $CoCl_2$, 0.001 was added at 40 mL per pot every week to ensure crop growth are not limited by these nutrients (Nuruzzaman et al. 2005b). In addition to the nutrient solution RO water was added regularly to maintain the soil at 70% WHC by weight.

Six weeks after planting, the wheat shoots were cut at the soil surface and rinsed with RO water. Wheat roots were carefully extracted from the soil and rhizosphere soil was obtained after shaking off the soil loosely adhering to the roots; the strongly adhering soil was collected by gentle brushing, and stored at -20 °C for P fractionation. Shoots and roots were dried at 70°C for 48 h, weighed and ground separately for total P and N analyses. The samples were digested in a nitric and perchloric acid mixture (6:1) for 5 h and total P was measured using the vanado-molybdate method (Hanson 1950). The digestion for plant total N was carried out using the Kjeldahl method (Bradstreet 1965) and the N concentration was measured colorimetrically (Bremner 1965).

For determination of soil P pools, sequential fractionation was carried out using the method described by Tiessen and Moir (1993) with slight modifications. Using mild to stronger extractants sequentially, the procedure removes the most labile P followed by less labile P forms (Tiessen and Moir 1993). Briefly, 1 g of sieved rhizosphere soil (<0.5 mm) was weighed and extracted sequentially with 30 mL of the following solutions: 1) water + anion exchange membrane (resin P); 2) 0.5 M $NaHCO_3$; 3) 0.1 M NaOH; 4) 1 M HCl and 5) 0.1 M NaOH. Another aliquot of the soil was used to measure microbial P using the resin method. Anion-exchange resin membrane (BDH #55164) was added to tubes with 30 mL of water and 1 mL hexanol as the fumigant. Phosphorus in the resin for both sets (fumigated and non-fumigated) was then eluted with 0.1 NaCl/HCl and the difference in P concentration between fumigated and non-fumigated soil was considered to be microbial P. The inorganic P (Pi) concentration in all extracts was determined using the molybdenum blue method (Murphy and

Riley 1962). Total P in the 0.5 M $NaHCO_3$ and 0.1 M NaOH fractions was determined by digestion of the extract with sulphuric and perchloric acid (6:1) for 10 h (Kuo 1996). Organic P (Po) in these fractions is the difference of total P and inorganic P fractions (Pi). Organic P in the HCl fraction was not determined as preliminary tests showed that the amount of organic P in this fraction is very low, which is in agreement with Tiessen and Moir (1993). Double extraction with 0.1 M NaOH was carried out as previous studies with this soil showed that there were significant amounts of NaOH-extractable P after the extraction with 1 M HCl. Residual P in the remaining soil and soil total P of the un-fractionated soil were determined by digesting the samples in nitric and perchloric acid mixture (6:1) for 4 h. Resin P and bicarbonate-extractable ($NaHCO_3$) Pi and Po are considered as labile pools (Tiessen and Moir 1993; Linquist et al. 1997). The NaOH-extractable Pi and Po, acid-extractable (HCl) Pi and residual P are less labile pools (Hedley et al. 1982; Tiessen and Moir 1993). The concentrations of the soil P pools prior to planting of wheat were only measured in soils without residue addition. Changes in P pools by the pre-crops were calculated by comparing the concentration of the P pools in the pre-crop soil with the unplanted control soil whereas changes in the rhizosphere of the following wheat were calculated by comparing the concentration of the P pools with the initial concentration of the soil of each pre-crop prior to planting of the wheat.

Statistical analysis

Statistical analysis based on four replicates per treatment in incubation study and three replicates in wheat growth experiment was performed by analysis of variance (ANOVA); post-hoc analyses (where appropriate) were performed using Tukey's multiple comparison test at $P \leq 0.05$ (GenStat® for Windows 11.0 ;VSN Int. Ltd, UK). Significant differences refer to $P \leq 0.05$. Correlation analysis was carried out using PASW, 17th edition.

Results

Incubation study: Respiration and changes in available P and N

The respiration rate was lowest in the unamended soil throughout the experiment (data not shown). In soils with added residues, the respiration rate peaked on day 1 and then decreased sharply until day 8 after which decreased more slowly until day 21 and then remained stable until day 42. The respiration rate on day 1 was highest in the soil with chickpea shoot residues with 1.1 mg CO_2-C g^{-1}soil day^{-1} and lowest in the soil with wheat root residues with 0.1 mg CO_2-C g^{-1} soil day^{-1}. Cumulative respiration on day 42 (Figure 2) was lowest in the unamended control soil, and ranged between 2.3 to 7.5 mg CO_2-C g^{-1} soil in the residue-amended soils, being highest in soil with chickpea shoots (Ps) and lowest with wheat root (Wr) residues. For the soils with chickpea and lupin residues, cumulative respiration was significantly higher with shoots than with root residues, whereas there were no differences between plant parts for faba bean and wheat residues. Cumulative respiration of the mixed root and shoots was between that of roots and shoots for chickpea, lupin and wheat, but not for faba bean.

The resin P concentration decreased significantly from day 0 to day 21 but then remained unchanged until day 42 in most treatments except for the unamended soil and the soil with wheat shoot residues (Ws) and mixed shoot and root residues (Wm) (Figure 3A) where it was higher on day 42 than on day 0. On day 42, compared to the unamended soil, addition of legume residues increased resin P concentrations 2.6 to 5.2-fold whereas they were reduced by addition of wheat residues. The resin P concentration was greatest with white lupin root (Lr) and lowest with wheat root residues (Wr); it was higher with shoot residues than that with root residues for faba bean, but the reverse was true for lupin and chickpea residues.

On day 0, the inorganic N concentration did not differ between residue-amended soils and the unamended control (Figure 3B). Compared to day 0, the inorganic N concentration on day

21 was significantly higher in the unamended soil and all treatments with legume residues except for soil with chickpea root residues (Pr) and mixed shoot and roots (Pm). In these treatments and the soil amended with wheat residues, the inorganic N concentration was lower on day 21 than on day 0. On day 42, the inorganic N concentrations were higher than on day 21 in most treatments except for the unamended soil and the soil with wheat root residues. Compared to the unamended control soil, inorganic N concentrations on day 42 were 32 to 109% higher in legume residue-amended soils except for chickpea root and mixed shoot and roots. On the other hand, the addition of wheat residues decreased inorganic N concentrations. Except for white lupin, the addition of legume shoot residues increased the inorganic N concentrations more than the addition of root residues.

On day 1, respiration rate was positively correlated with resin P ($r = 0.59$, $P < 0.01$) Cumulative respiration was positively correlated with concentrations of resin P and inorganic N in soil on day 21 ($r = 0.49$, $P<0.01$ and 0.30, $P<0.05$) and on day 42 ($r = 0.59$, 0.53, $P<0.01$). Cumulative respiration on day 42 was negatively correlated with both C/N and C/P ratio of the residues ($r = -0.651$, -0.604, $P<0.01$).

Wheat growth in pre-crop soil: Wheat biomass, P uptake and N concentration

Wheat grown in the previously unplanted control soil had the greatest root and shoot biomass. Wheat biomass was higher when grown in legume pre-crop soil without residues and the unplanted control soil than in residue-amended soil (Table 2). In the pre-crop soils without residues, wheat biomass was greatest in faba bean pre-crop soil and lowest in soil previously cropped with chickpea. In the soils with residue addition, wheat biomass was greatest in the white lupin pre-crop soil with root residues and lowest in chickpea pre-crop soil with root residues. Compared to the pre-crop soil without residues, the addition of residues decreased wheat biomass by about 50% with no clear differences between the effect of shoots and roots except in the white lupin pre-crop soil where the addition of shoot residues decreased biomass more than the addition of root residues.

There were no significant differences among the treatments in wheat shoot P concentration which ranged from 2.93 to 3.64 mg g^{-1} (Table 2). The root P concentration was greatest in wheat grown in the faba bean pre-crop soil with root residues and lowest in the faba bean pre-crop soil without residues. Wheat grown in the previously unplanted control soil had the greatest P uptake which was significantly greater than in wheat grown in legume pre-crop soil with or without residue addition. The lowest P uptake was found in wheat grown in the chickpea pre-crop soil with shoot and root residues. Phosphorus uptake of wheat grown in legume pre-crop soils was reduced by residue addition except when grown in white lupin pre-crop soil with root residues.

The shoot N concentration of wheat grown in the residue-amended soils was significantly greater than that in the previously unplanted control soil or in the legume pre-crop soils without residues (Table 2). It was greatest in wheat grown in the soil with chickpea shoot residues and lowest in wheat grown in the chickpea and faba bean pre-crop soils. The root N concentration was greatest in wheat grown in the white lupin pre-crop soil with shoot residues and lowest in wheat grown in the previously unplanted control soil.

Size and changes of P pools in pre-crops and the rhizosphere soil of following wheat

Compared to the unplanted control soil, the resin P concentration prior to the planting of wheat was 38 to 68 % lower in the soil previously cropped with legumes, being lowest in the soil previously planted with faba bean (Table 3). The concentration of $NaHCO_3$-Pi was greatest in the previously unplanted control soil. The concentrations of HCl-Pi and residual P were higher in the soil previously grown with faba bean than in the other pre-crop soils. The concentration of $NaHCO_3$-Po was lowest in soil previously cropped with chickpea, while the concentrations of NaOH-Pi and NaOH-Po did not differ among the pre-crop soils. During the pre-crop phase, compared to the unplanted soil, microbial P was depleted by white lupin but increased by faba bean (Table 4A). The pre-crops also depleted resin P, $NaHCO_3$-Pi with the greatest depletion by faba bean. Relative to the unplanted soil, only chickpea depleted

$NaHCO_3$-Po, whereas NaOH-Pi was depleted only by white lupin. All pre-crops resulted in an increase in residual P compared to the unplanted soil whereas only faba bean increased HCl-Pi. Except for faba bean pre-crop soil, the sum of decreases was greater than the sum of increases.

After 6 weeks, the concentration of microbial P in the rhizosphere of wheat was similar as in the unplanted control soil. Among the pre-crop treatments, microbial P in the rhizosphere of wheat grown in the chickpea pre-crop soil with shoot and root residues was higher than in the other pre-crop soils (Figure 4A). Compared to the unplanted soil, the resin P concentration was, irrespective of residue addition, lowest in wheat grown in the faba bean pre-crop soil and highest in wheat grown in the white lupin pre-crop soil. In all pre-crop soils, the concentration of resin P was lower in the rhizosphere of wheat grown in pre-crop soil without residues than with residue addition. The concentration of $NaHCO_3$-Pi was significantly lower in the rhizosphere than in the unplanted control soil except in the rhizosphere of wheat grown in white lupin pre-crop soil with shoot residues (Figure 4B). The $NaHCO_3$-Pi concentration in the rhizosphere of wheat grown in pre-crop soil was increased by residue addition particularly in chickpea and white lupin pre-crop soils. The $NaHCO_3$-Pi concentration was generally lower in wheat grown in the soil previously cropped with faba bean compared to wheat in chickpea and white lupin pre-crop soil. Compared to the unplanted control, the concentration of NaOH-Pi was significantly lower in the rhizosphere of wheat grown in the soil previously cropped with faba bean and chickpea regardless of residue addition (except chickpea root)(Figure 4C). There were no significant differences among the treatments in concentrations of the $NaHCO_3$-Po, NaOH-Po, HCl-Pi and residual P (Figure 4B, C &D).

Relative to the concentrations prior to the planting of wheat, microbial P was significantly decreased in the rhizosphere of wheat grown in the soil previously cropped with faba bean and chickpea while it increased in wheat grown in the previously unplanted soil and white lupin pre-crop soil (Table 4B). Resin P, NaOH-Pi and Po and residual P were depleted in

rhizosphere of the wheat, whereas there was an accumulation of $NaHCO_3$-Po. Compared to the concentrations prior to the planting of wheat, wheat grown in faba bean and chickpea pre-crop soils depleted $NaHCO_3$-Pi. The HCl-Pi concentration was decreased in the rhizosphere of wheat grown in faba bean pre-crop soil, whereas it was increased in wheat grown in the previously unplanted soil and white lupin pre-crop soils.

In the rhizosphere of the following wheat, the sum of decreases was greater than the sum of increases, with the strongest decrease in wheat grown in the soil previously planted with faba bean and the smallest decrease in wheat grown in white lupin pre-crop soil. Thus, there was a net decrease in P compared to the soil prior to planting.

There were no significant relationship between the changes of P pools by the pre-crops and the following wheat. Wheat P uptake was positively correlated with the concentration of microbial P, $NaHCO_3$-Pi, NaOH-Pi as well as HCl-Pi ($r = 0.80$, 0.59, 0.78 and 0.65, $P \leq 0.05$; respectively). A stepwise multiple regression of wheat P uptake with the concentration of the rhizosphere P pools resulted in the equation:

$$\text{P uptake} = 1.53 - 0.36 \text{ microbial P} + 0.15 \text{ NaOH-Pi}$$

Discussion

The results of this study showed that the addition of legume residues to soils previously cropped with legumes had a negative effect on the early growth and P uptake of the following wheat although N and P were released during residue decomposition. With respect to the changes in P pools, all crops (legume pre-crops and wheat) depleted resin P, but the effect on the other P pools differed among crops. Whereas the legume pre-crops depleted $NaHCO_3$-Pi and Po as well as NaOH-Pi, there was an accumulation of residual P. Similarly, wheat depleted NaOH-Pi, but also NaOH-Po and residual P. On the other hand, there was an accumulation of $NaHCO_3$-Po in the rhizosphere of wheat. Thus, this study showed that

legumes and wheat have access to labile and less labile P pools with the effect of wheat on the P pools modulated to some degree by the pre-crop.

Legume residue decomposition

In the incubation experiment, the addition of most legume residues resulted in net P and N mineralization except for chickpea root residues which immobilised N (Figure 3A, B). On the other hand, the addition of wheat residues resulted in net immobilisation of both P and N. It has been suggested that a C/P ratio below 300 results in net P mineralisation (Brady and Weil, 2002). Thus, the increase in resin P with legume residue addition can be explained by their lower C/P ratio (<250) compared to wheat (>440). Net N mineralisation can be expected at C/N ratios less than 30 (Parnas 1975; Brady and Weil 2002). Therefore, the net N mineralisation with most legume residues can be explained by their low C/N ratio which was less than 30 whereas that of wheat was greater than 40. Chickpea shoot residues decomposed faster than the other legume residues although they had the highest C/N ratio whereas faba bean shoot and white lupin shoot residues had a lower C/N ratio and decomposed more slowly. This suggests that the higher decomposition rate of chickpea shoot residues can be, in part, be explained by its lower C/P ratio which was 200 compared to 230-250 in faba bean root and white lupin shoot residues. However, other residues decomposed more slowly than chickpea shoot residues although they had lower C/N and C/P ratios, e.g. faba bean shoots. This indicates that decomposition rate of residues is influenced by residue properties other than nutrient ratios such as lignin and polyphenol concentrations (Wichern et al. 2007) which are negatively correlated with decomposition rate (Tian et al. 1992).

Wheat biomass and P pools

Wheat biomass was greater in the previously unplanted control than in the legume pre-crop soils irrespective of residue addition (Table 2), which is in agreement with Kamh et al. (1999) and Nuruzzaman et al. (2005a). During the pre-crop phase, the unplanted soil had received the

same nutrient additions as the legume pre-crops. Therefore, the high growth and P uptake of wheat grown in the previously unplanted control soil could be due to the greater amount of nutrients including P still remaining in the soil compared to the pre-crop soils where the nutrients were taken up by the legumes. Indeed, the resin P concentration remained highest in the rhizosphere of wheat grown in the previously unplanted control soil (Figure 5A), despite the greatest depletion of this pool (Table 4) compared to soil prior to planting. Among the legume pre-crop soils, wheat biomass in the faba bean pre-crop soil was greater than with other legumes, which is in agreement with Nuruzzaman et al. (2005a, b)

Although the incubation study showed that there was a net release of N and P with addition of legume residues, wheat growth was reduced by residue addition. This can be explained by several factors: (i) due to the lower concentration of residues added in the pot experiment (8 g kg^{-1}) than in the incubation study (20 g kg^{-1}), less N and P would have been released, (ii) the small root system of the young wheat plants would not have been able to access all N and P released from the residues, (iii) the net N and P release in the incubation study was measured after 21 days, but it cannot be ruled out that there was a net N and P immobilisation initially which would coincide with high nutrient demand by the wheat, and (iv) toxic compounds can be released during the decomposition of the legume residues such as phenyl-acetic, 4-phenylbutyric, salicylic, benzoic and o-hydroxypenylacetic acids which can inhibit plant growth (Chou and Patrick 1976). At later stage of crop growth, P may become available by microbial turnover or/and P release from decomposing residue as its C/P and C/N ratio decreases due to loss of C via respiration. This negative effect of residue addition is in contrast to other studies which reported positive effects of legumes crop residues on the growth of following cereals grown for a similar or longer duration compared with our study (Hafner et al. 1993; Horst et al. 2001; Kamh et al. 2002; Nuruzzaman et al. 2005a) which were attributed to the recycling of P through residue addition (Horst et al. 2001; Nuruzzaman et al, 2005a). This suggests that the negative effect of residue addition to the

following wheat growth found in our study is unlikely to be due to short duration of wheat growth alone.

Prior to planting of the legume pre-crops, the soil was fertilized with 80 mg P kg^{-1} as KH_2PO_4 (Mat Hassan et al. 2011). This may explain why the depletion of labile pools, resin P and bicarbonate-extractable P by the pre-crops was greater than by the following wheat since these pools are the principle sources of P for legumes when P availability is high (Vu et al. 2008; Mat Hassan et al. 2011).

The effects of the crops on the organic P pools were markedly different. Whereas the concentrations of the labile $NaHCO_3$-Po pool and the less labile NaOH-Po pools were little affected by the legume pre-crops, there was an accumulation of $NaHCO_3$-Po and a depletion of NaOH-Po in the rhizosphere of wheat. The lack of change of the organic P pools by the legumes may be due to the high availability of inorganic P in the pre-crop phase. The differential effect of wheat on the two organic P pools and the depletion of NaOH-Pi and residual P suggest that wheat has access to less labile P pools if the concentration of available P is low. Phosphorus in these P pools can be released by carboxylates (Pi) and phosphatases (Po) (Gerke 1992; Richardson et al. 2001; George et al. 2002; Wang et al. 2006). Previous studies have shown that compared to legumes, wheat released relatively small quantities of carboxylates (Pearse et al. 2006). However, in agreement with the results of the present sudy, Rose et al. (2010) found that in an alkaline soil wheat depleted NaOH-Pi which is P associated with Al and Fe hydrous oxide (Hedley et al. 1982). Rose et al. (2010) suggested that the depletion of this pool may be due to (i) nitrate uptake which leads to increased rhizosphere pH, or (ii) equilibrium between NaOH-Pi and $NaHCO_3$-Pi leading to dissolution of NaOH-Pi when the $NaHCO_3$-Pi is depleted. The former suggestion is unlikely to be relevant for our study as the pH of the bulk soil during the pre-crop growth (Mat Hassan et al. 2011) and the following wheat was decreased (data not shown). Therefore, depletion of $NaHCO_3$-Pi by wheat followed by release of P from NaOH-Pi to regain the equilibrium

between the two pools is the more likely reason for the depletion of this less labile pool. Although the depletion of some pools in the rhizosphere of wheat was accompanied by increases in other P pools, e.g. $NaHCO_3$-Po, the sum of decreases was greater than the sum of increases which confirms the ability of wheat to deplete soil P.

The greater depletion of residual P in the rhizosphere of wheat grown in the pre-cropped soils compared to the previously unplanted soil and the greater depletion of NaOH-Pi and HCl-Pi in wheat after faba bean could be due to residual carboxylates and phosphatases released by the legumes maintaining at least part of their activity. Although carboxylates can be rapidly decomposed in soil (Ström et al. 2001; Jones et al. 2003), their decomposition is inhibited by sorption to soil particles (Jones 1998; Jones and Edwards 1998; Jones et al. 2003) which is also the case for phosphatases (George et al. 2005). The depletion of residual P [P strongly bound to Al and Fe oxides (Hedley et al. 1982)] by wheat could be due to either direct access by wheat or, more likely, the dissolution of P from this less labile pool into labile P pools which were depleted by wheat to regain an equilibrium. Changes in residual P have been shown previously, with increases as well as decreases being reported depending on soil type (Hedley et al. 1982; Wang et al. 2007), rhizosphere acidification (Vu et al. 2008) and plant P acquisition strategies (Nuruzzaman et al. 2005b).

Conclusions

This study showed that although addition of legume residues increased available P compared to wheat residues, the presence of legume residues negatively affected the growth of the following wheat. Among the legumes, faba bean pre-crop stimulated wheat growth most and induced the greatest depletion of P pools in the rhizosphere of wheat, suggesting that faba bean is a promising legume for crop rotation practices in alkaline soils. The experiment was carried out for duration of 6 weeks and future research on the effect of these legumes on grain yield and nutrient uptake up to maturity is needed to validate the findings from this present study. Furthermore, enzyme activities and carboxylate concentrations need to be determined

prior to wheat growth to further elucidate the mechanisms by which the soil P pools are accessed during the growth of the following wheat.

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Table 1 Properties of the plant residues.

Residues	Plant part	C	N (mg g^{-1})	P	C: N ratio	C:P ratio
Faba bean	shoot	411	24.6	2.3	16.7	176
	root	386	19.3	1.6	20.0	249
Chickpea	shoot	414	15.3	2.1	27.1	201
	root	376	19.3	2.6	19.4	144
White lupin	shoot	434	23.0	1.9	18.8	232
	root	200	09.4	1.6	21.2	126
Wheat	shoot	429	07.4	0.8	58.3	545
	root	362	08.5	0.8	42.8	445

Table 2: Biomass (dry weight), concentrations of P and N, and P uptake of the following wheat grown for 6 weeks in a previously unplanted control soil, legume pre-crop soils (faba bean, chickpea and white lupin) and pre-crop soils with residue addition (shoots and roots) (n=3). Values in the same column followed by the same letter are not significantly different ($P \leq 0.05$) by Tukey's multiple comparison tests.

Wheat	Biomass (g dw pot^{-1})		P concentration (mg g^{-1})		N concentration (mg g^{-1})		P uptake (mg pot^{-1})
	shoot	root	shoot	root	shoot	Root	
W-control	0.68 f	0.56 e	3.22 a	1.31 ab	12.9 ab	7.2 a	5.33 f
W-faba bean	0.61 ef	0.38 cd	3.07 a	1.13 a	11.0 a	9.0 a	3.86 e
W-faba bean shoot	0.30 b	0.22 ab	3.61 a	1.83 abc	30.3 cd	26.6 bcd	2.53 b
W-faba bean root	0.31 bc	0.23 abc	3.31 a	2.34 c	24.5 abcd	25.6 bc	2.79 bc
W-chickpea	0.42 d	0.30 bcd	2.93 a	1.67 abc	11.0 a	11.3 a	3.03 bcd
W-chickpea shoot	0.23 ab	0.14 a	2.93 a	1.92 abc	34.5 d	27.2 bcd	1.50 a
W-chickpea root	0.21 a	0.13 a	3.15 a	2.18 abc	28.1 bcd	33.4 cd	1.52 a
W-white lupin	0.59 e	0.35 bcd	3.00 a	1.17 a	14.0 ab	10.0 a	3.66 de
W-white lupin shoot	0.19 a	0.14 a	3.57 a	1.91 abc	32.0 d	39.0 d	1.53 a
W-white lupin root	0.40 cd	0.41 de	3.64 a	1.51 abc	16.0 abc	16.2 ab	3.87 cde

Table 3: Concentration of different P pools (mg P kg^{-1} soil) in the bulk soil of the unplanted control soil and legume pre-crop soils (faba bean, chickpea and white lupin) prior to the addition of residues and planting of wheat (n=4). Values in the same column followed by the same letter are not significantly different ($P \leq 0.05$) by Tukey's multiple comparison tests.

	Microbial P	Resin P	$NaHCO_3$		NaOH		HCl-Pi	Residual P
			Pi	Po	Pi	Po		
			mg P kg^{-1} soil					
Control	4.9 b	55.8 d	15.6 c	2.8 b	19.7 b	78.0 a	20.8 a	136.7 a
Faba bean	6.3 c	17.9 a	9.1 a	2.5 b	19.0 ab	74.1 a	31.1 b	182.6 c
Chickpea	4.0 b	22.6 b	9.2 a	1.3 a	17.7 ab	78.1 a	22.2 a	156.1 b
White lupin	1.3 a	34.7 c	12.3 b	2.7 b	16.7 a	76.1 a	20.4 a	152.9 b

Table 4: (A) Changes in P pools relative to the unplanted control soil induced by legume pre-crops prior to planting of wheat and (B) changes in P pools in the rhizosphere soil of following wheat grown for 6 weeks in the unplanted control soil and legume pre-crop soils (faba bean, chickpea and white lupin) relative to the P concentrations in the bulk soil of each pre-crop prior to planting (n=3). Negative values indicate decreases while positive values increases. * indicates significant change compared to the control soil (section A) while values in the same column followed by the same letter are not significantly different (section B) ($P \leq 0.05$) by Tukey's multiple comparison tests.

Treatments	changes in P pools (mg P kg^{-1})									
	Microbial P	Resin P	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-Pi	Residual P	Sum of decreases	Sum of increases
A:changes by legume pre-crops										
Faba bean	1.4 *	-38.0 *	-6.6 *	-0.3	-0.7	-3.9	10.3 *	45.9 *	-49.5	57.7
Chickpea	-0.9	-33.2 *	-6.5 *	-1.5 *	-2.0	0.1	1.4	19.5 *	-44.1	20.9
White lupin	-3.6 *	-21.1 *	-3.4 *	-0.1	-3.0 *	-1.9	-0.3	16.3 *	-33.3	16.3
B: changes by the following wheat										
W-control	0.8 cd	-14.9 a	1.3 c	22.2 a	-3.0 cd	-17.3 a	3.3 bc	-76.9 c	-112.1	27.6
W-faba bean	-2.8 ab	-6.8 bc	-1.6 a	11.5 a	-8.2 ab	-25.9 a	-9.7 a	-110.1 a	-165.0	11.5
W-chickpea	-0.5 bc	-7.5 b	-1.4 ab	10.4 a	-6.9 b	-21.4 a	-0.2 abc	-89.0 bc	-127.1	10.4
W-white lupin	2.5 d	-6.8 bc	0.4 bc	23.5 a	-1.5 d	-21.8 a	8.7c	-85.5 bc	-115.6	35.0

[A] Difference in concentration of a given P pool between the bulk soil of legume pre-crops and the unplanted control soil

[B] Difference in concentration of a given P pool between the rhizosphere soil of wheat grown in legume pre-crop soil and the concentration prior to planting of the wheat

List of figures

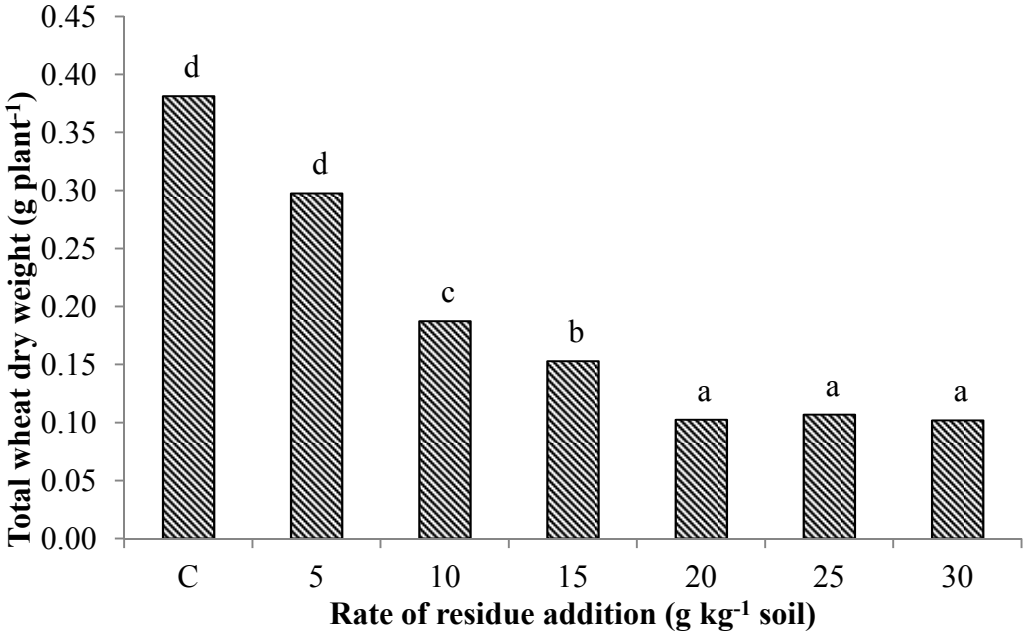

Figure 1

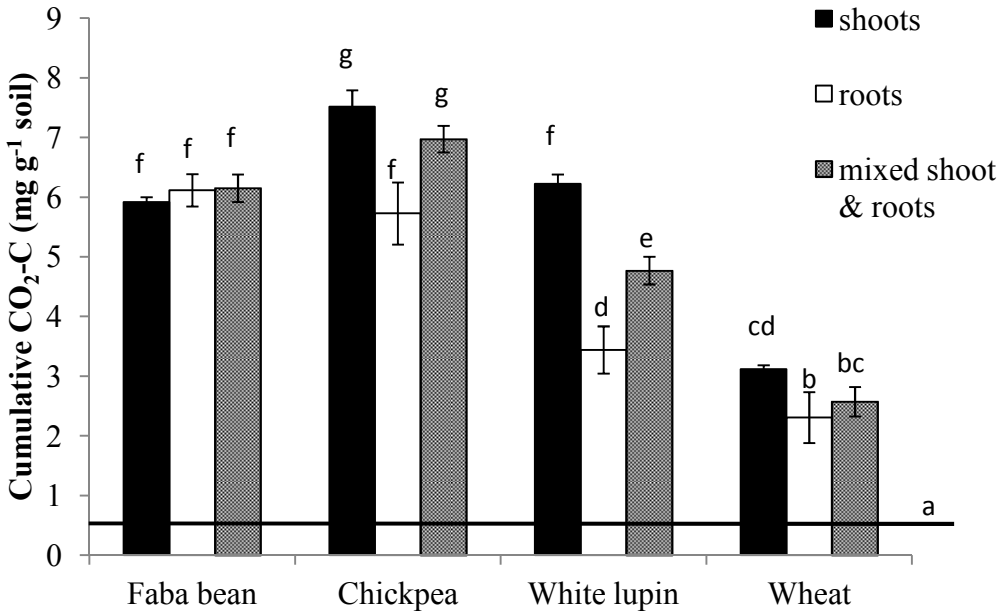

Figure 2

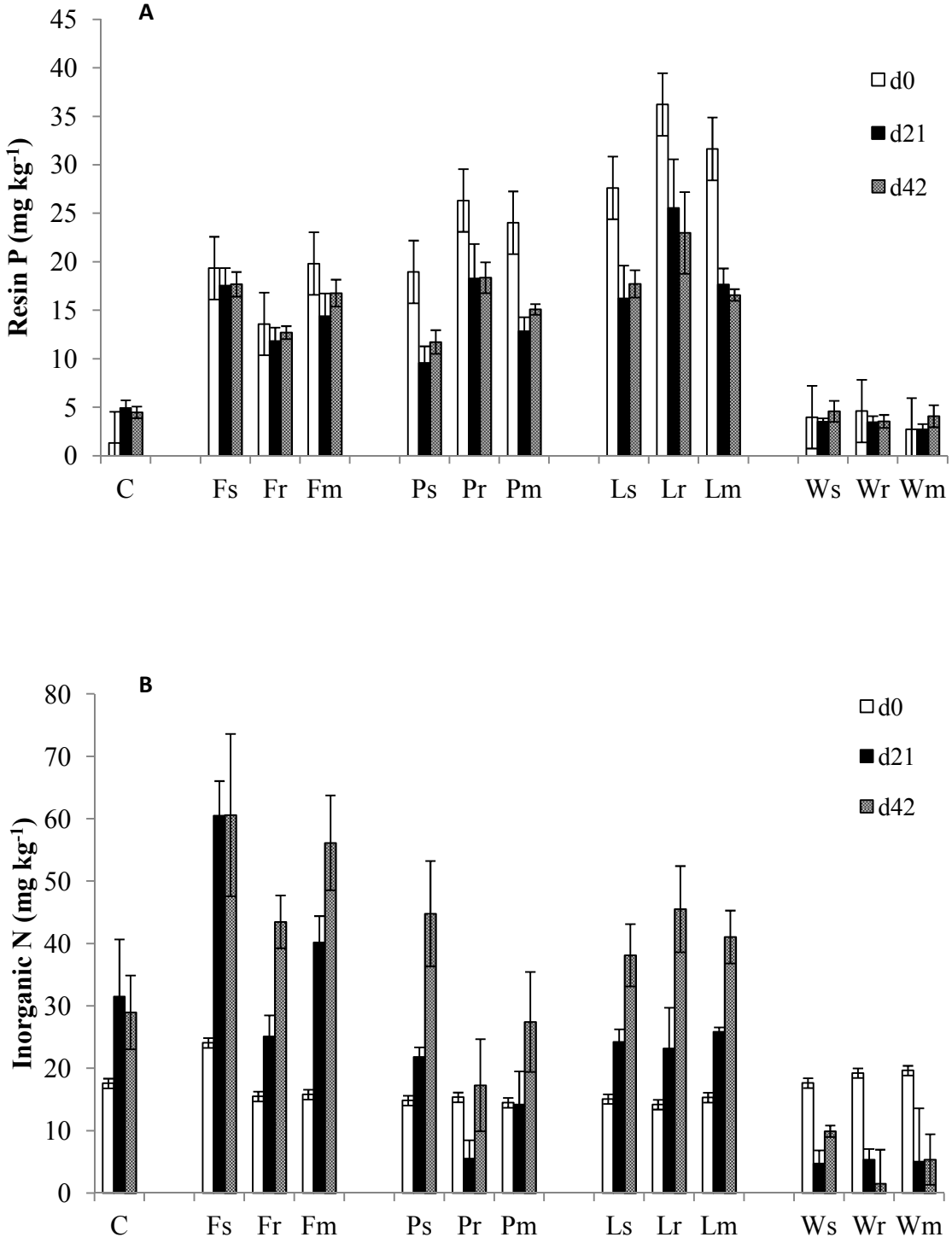
A
Resin P (mg kg-1)
45
40
35
30
25
20
15
10
5
0
d0
d21
d42
C
Fs
Fr
Fm
Ps
Pr
Pm
Ls
Lr
Lm
Ws
Wr
Wm
B
Inorganic N (mg kg-1)
80
70
60
50
40
30
20
10
0
d0
d21
d42
C
Fs
Fr
Fm
Ps
Pr
Pm
Ls
Lr
Lm
Ws
Wr
Wm

Figure 3

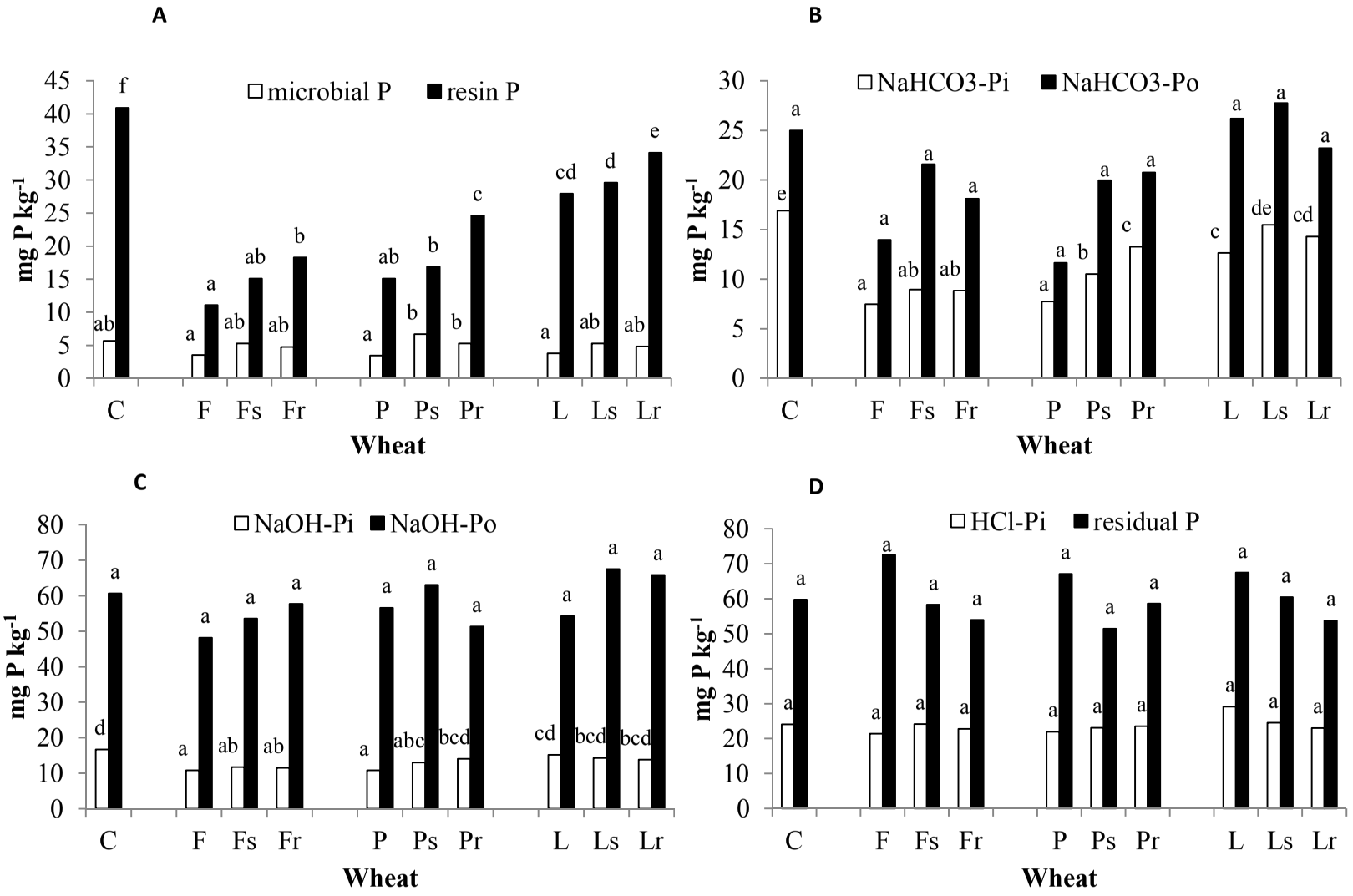
A
microbial P
resin P
mg P kg-1
Wheat
C F Fs Fr P Ps Pr L Ls Lr
B
NaHCO3-Pi
NaHCO3-Po
mg P kg-1
Wheat
C F Fs Fr P Ps Pr L Ls Lr
C
NaOH-Pi
NaOH-Po
mg P kg-1
Wheat
C F Fs Fr P Ps Pr L Ls Lr
D
HCl-Pi
residual P
mg P kg-1
Wheat
C F Fs Fr P Ps Pr L Ls Lr

Figure 4

Chapter 4

Rotation of grain legumes and wheat in acidic soil – plant growth, P uptake and rhizosphere P pools

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Rotation of grain legumes and wheat in acidic soil –plant growth, P uptake and rhizosphere P pools

Plant and Soil; Revised manuscript

Mat Hassan, H (Candidate)

Performed analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author.

I hereby certify that the statement of contribution is accurate.

Signed Date

Tang, Caixian

Manuscript evaluations

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date

Marschner, P

Supervised development of work, data interpretation and manuscript evaluation.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date

Rotation of grain legumes and wheat in acidic soil –plant growth, P uptake and rhizosphere P pools

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Abstract

Background Some legumes have been shown to deplete less labile P pools and enhance the growth of cereals in either intercropping or crop rotation. To date, there is little information on how the changes in the soil P pools induced by legumes affect growth and P uptake and the size of the P pools in the rhizosphere of the following wheat.

Methods A glasshouse experiment was carried out with a crop rotation of wheat and three grain legumes in the first phase followed by wheat in the second phase. At the end of each phase, crop growth, P uptake and rhizosphere P pools were measured. Wheat (*Triticum aestivum* L.) and nodulated faba bean (*Vicia faba* L.), white lupin (*Lupinus albus* L.) and yellow lupin (*Lupinus luteus* L.) were grown in an acidic sandy loam soil (pH_{water} 5.4) low in available P to which 100 mg P kg^{-1} was added and harvested at pod set of the legumes. This phase also included unplanted pots. Then, pre-crop soils with and without residues were planted with wheat which was grown for 6 weeks. Sequential P fractionation was used to determine the concentration of different soil P pools.

Results In Phase 1 of the rotation, growth of faba bean and white lupin was greater than of yellow lupin and was lowest in wheat. Compared to the unplanted soil, the depletion of labile and less labile P pools was greatest in the rhizosphere of white lupin despite higher P uptake by faba bean. In the following phase, wheat growth decreased in the following order: previously unplanted soil > wheat pre-crop > legume pre-crops. The addition of legume and wheat residues decreased the growth of the following wheat, but increased the concentration of most P pools in the rhizosphere, particularly organic pools of the $NaHCO_3$, NaOH and concentrated HCl fractions.

Conclusions This study confirmed the greater ability of white lupin to mobilise labile and less labile P pools compared to other legumes and wheat. The addition of most pre-crop residues had a negative effect on the early growth and P uptake but increased the concentration of organic P pools in the rhizosphere of the following wheat.

Keywords

Crop rotation, Grain legumes, Pre-crops, P fractionation, P uptake, Wheat.

Introduction

In most farming systems, addition of P fertilisers is crucial to ensure sustainable crop production. After nitrogen, P is the second most limiting nutrient for crop growth (Vance 2001). High-grade rock-P deposits which are used to produce P fertilisers are estimated to be depleted within the next 50 to 100 years (Cordell et al. 2009).

Plants and microorganisms take up P from the soil solution but the majority of soil P is not immediately available (Sanyal and De Datta 1991). During the first year, plants take up about 20-40% of the applied P (Vance 2001) while the remaining P is rapidly adsorbed to the soil constituents rendering P poorly available for plants (Richardson et al. 2001; Vu et al. 2008). Therefore, in order to maximise yield, farmers frequently add more P than required by the

crop. This has resulted in the build up of 'less available' soil P pools which can potentially be accessed by P-efficient plant such as legumes (Nuruzzaman et al. 2006).

The effects of legumes on other crops particularly cereals in crop rotation or intercropping has been studied extensively. In addition to improved N availability by N_2 fixation, legumes also positively influence the growth of subsequent cereal crops where N was not limiting cereal growth (Asseng et al. 1998). The positive effects by legume pre-crops are thought to be due to improved soil P availability to the following crops (Alvey et al. 2001; Kamh et al. 2002; Nuruzzaman et al. 2005b), changes in soil physical properties (Horst and Härdter 1994; Obi 1999), higher mycorrhizal colonisation and the suppression of diseases (Bagayoko et al. 2000), stimulation of microbial activity in the rhizosphere (Marschner et al. 2004) and nutrient release during the decomposition of the legume residues (Kamh et al. 2002). Some legumes have a greater ability to mobilise less-labile P compared to cereals which can be explained by (i) modifications of the rhizosphere soil pH due to proton efflux or influx (Tang et al. 1998; Hinsinger et al. 2003), (ii) exudation of organic acid anions by cluster and non cluster-rooted species (Neumann et al. 1999; Neumann and Römheld 1999; Wouterlood et al. 2004) and, (iii) increased phosphatase activity to mineralise soil organic P (Li et al. 2003; Li et al. 2004). The ability to mobilise soil P can increase P uptake and therefore the P concentration in the legume residues.

Most published studies show increased growth of cereals following legumes. However in our previous study in an alkaline soil under non P limiting conditions, legume pre-crops had a negative effect on growth and P uptake of the following wheat and residue addition further decreased wheat growth (Mat Hassan et al. 2012a). Further, although pre-crop legumes differed in depletion of P pools, there were little differences among wheat grown in soil that had been planted with different pre-crops (Mat Hassan et al. 2012b). In contrast to alkaline soils, P is predominately associated with Fe and Al oxides in acidic soils. The release of P from these pools is based on different mechanisms than for Ca associated P dominating in

alkaline soils. This may affect the ability of legumes and wheat to deplete P pools and also the effect of pre-crops on the following wheat. Therefore, the aims of the present study were (i) to determine growth, P uptake and the size of P pools in the rhizosphere soil of grain legume and wheat pre-crops in an acidic soil with P addition, (ii) to compare the effect of the pre-crop legumes and wheat on the growth, P uptake and the size of the P pools in the rhizosphere of the following wheat and, (iii) to determine whether these effects are modulated by the addition of pre-crop residues.

Materials and Methods

A sandy loam soil (0-10 cm) under natural vegetation was collected in Mt Bold (latitude 35° 04' 21.60 S, longitude 138° 42'43.80 E, elevation 402 m), South Australia. The soil was air-dried and passed through a 2-mm sieve. Soil pH was measured after shaking in a 1:5 soil/water ratio for 1 h. Resin P was determined using an anion-exchange resin membrane (BDH #55164) (Kouno et al. 1995). Total P was determined by wet digestion of a mixture nitric and perchloric acid (6:1) (Kuo 1996) and the P concentration was determined colorimetrically using the molybdenum blue method (Murphy and Riley 1962). Available N was extracted with 2 M KCl as described by Keeney and Nelson (1982). Nitrate was measured by the cadmium reduction method (Henrikson and Selmer-Olsen 1970), while ammonium was measured by the nitroprusside/dichloro-S-triazine modification of the Berthelot indophenol reaction (Searle 1984). Field capacity was measured by the hanging column method (Klute 1986). The P-buffering index (PBI) was measured according to Rayment and Lyons (2010). The properties of the soil are as follows: pH (1:5 soil:water) 5.4, clay 19.9%, sand 52.6%, silt 27.5%, organic C 44 g kg^{-1}, total N 4.2 g kg^{-1}, total P 313 mg kg^{-1}, resin P 10.3 mg kg^{-1}, NO_3-N 6.2 mg kg^{-1}, NH_4-N 4.2 mg kg^{-1}, bulk density 1.43 g cm^{-3}, water holding capacity 300g kg^{-1} and P-buffering index 95.

Plant growth conditions

Phase 1: Growth of wheat and legume pre-crops

A preliminary experiment was conducted with faba bean and wheat over six weeks to determine the amount of added P required for maximal growth, with P additions ranging from 5 to 200 mg P kg^{-1} as KH_2PO_4. Maximal growth was reached at 100 mg P kg^{-1}; therefore, this P addition rate was used in the experiment.

The plants were grown in a glasshouse between late April to July (autumn to winter in the southern hemisphere). The temperature in the glasshouse ranged from 15 to 30ºC. The soil was carefully mixed with 100 mg P kg^{-1} as KH_2PO_4 and placed in 1.9 L pots lined with a polyethylene bag. Four pre-germinated seeds of faba bean (*Vicia faba* L. cv. Fiesta) (FB), yellow lupin (*Lupinus luteus* L.cv. Wodjil) (YL), white lupin (*Lupinus albus* L.cv. Luxor) (WL) and wheat (*Triticum aestivum* L.cv Krichauff) (W) were sown per pot (n=4). The weight per seed (g) at sowing was: faba bean 0.66, white lupin 0.32, yellow lupin 0.15 and wheat 0.04. There were also unplanted pots representing the bulk soil. Five days after sowing, the legumes were inoculated with *Bradyrhizobium* strain WU425 (group G) for yellow lupin and white lupin and *Rhizobium* strain WSM1455 (group F) for faba bean. Wheat was supplied with NH_4NO_3 at 80 mg N kg^{-1}. There were four replicate pots per treatment (4 plant species and unplanted control). Two weeks after sowing, the plants were thinned to two plants per pot. The soil was kept at 70% field capacity by adding reverse osmosis (RO) water by weight throughout the experiment. Basal nutrients, except N and P were supplied once a week as described in Nuruzzaman et al. (2005a). The composition of the nutrient solution was (µM): $CaCl_2$, 150; K_2SO_4, 100; $MgSO_4$, 54; H_3BO_3, 2.4; $MnSO_4$, 0.24; $ZnSO_4$, 0.1; Na_2MoO_4, 0.03; $CuSO_4$, 0.02; $CoCl_2.6H_2O$, 0.001. The nutrient solution was added at 100 mL per pot per week; after four weeks the volume was increased to 200 mL per pot weekly until the end of the experiment. The control pots received the same amount of nutrients. The pots were arranged in a complete randomised design. The legumes were harvested at pod set at which time wheat was at grain filling (Feekes scale 10.1, Reuter and Robinson 1997); the harvesting time varied slightly with plant species, but was at approximately 70 days after sowing. At

harvest, the roots were carefully removed from the soil, then the soil from each treatment was air-dried and kept for 3 months prior to the next phase of the rotation. The harvesting procedure, sampling of the rhizosphere soil and analyses of plant materials and soils are described below.

Phase 2: Growth of the following wheat

In the second phase, four pre-germinated wheat seeds (cv Krichauff) were grown in previously unplanted control soil, pre-crop soils (soil previously cropped with faba bean, white lupin, yellow lupin and wheat) without residue addition and pre-crop soils with residue addition (a 1:1 mixture of vegetative parts of shoots and roots). Ground and sieved residues (0.25 to 2 mm) (Table 1) were added at 20 g kg^{-1} and thoroughly mixed with the pre-crop soils prior to planting. The treatments (n=4) were as follows: unplanted control soil (W-C); white lupin pre-crop soil (W-L) with and without residues; faba bean pre-crop soil (W-F) with and without residues; yellow lupin pre-crop soil (W-Y) with and without residues and wheat pre-crop soil (W-W) with and without residues. Five days after sowing, the plants were thinned to 2 plants per pot. The pots were maintained at 70% field capacity by adding RO water to weight throughout the experiment. Nitrogen was supplied weekly as NH_4NO_3 at 2.4 mg N kg^{-1} and basal nutrients except P (as described for phase 1), were supplied at 30 mL per pot per week. The plants were harvested 6 weeks after sowing. Prior to the start of the second phase, the bulk soil of each pre-crop was analysed for P pools and these concentrations were compared to those in the rhizosphere of the following wheat to assess whether pre-crop affect the ability of wheat to access the soil P pools The sampling of the rhizosphere soil, harvesting procedure and analyses of plant materials and soils are described below.

Plant and soil analyses

At harvest of both phases, the shoots (including seeds) were cut at the soil surface, rinsed in RO water and then oven-dried at 80ºC for 48 h. The roots were gently removed from the pots

and soil loosely adhering to the roots was shaken off. The remaining tightly adhering soil, the rhizosphere soil, was collected by gentle brushing, and stored at -20°C for analysis of soil total P and P fractionation. There was approximately 10 to 15 g of rhizosphere soil per pot. The roots were then rinsed in RO water and oven-dried at 80ºC for 48 h. Shoots and roots were ground separately and digested in nitric and perchloric acid mixture (6:1) for 5 h, total P was measured using the vanado-molybdate method (Hanson 1950). Plant P uptake was calculated by multiplying P concentration by dry weight minus seed P content. Total C and N of the plant material were determined by high temperature combustion using a Leco CNS analyser. For determination of soil P pools, sequential fractionation was carried out using the method described by Tiessen and Moir (1993) with slight modifications. Fractionation removes the most labile P followed by more stable or less labile P forms by using mild to stronger extractants sequentially (Tiessen and Moir 1993). Briefly, 1 g of sieved rhizosphere (Phase 1 and 2) and bulk soil (Phase 1)(<0.5 mm) was extracted sequentially with 30 mL of the following solutions: 1) 0.5 M $NaHCO_3$; 2) 0.1 M NaOH; 3) 1 M HCl; 4) 0.1 M NaOH and 5) concentrated HCl. The second extraction with 0.1 M NaOH was carried out as previous studies showed that significant amounts of P were hydroxide-extractable after the extraction with 1 M HCl (Mat Hassan et al. 2012). Resin P and microbial P which were not included in the sequential fractionation were determined in a separate aliquot of soil using anion-exchange resin membrane (BDH #55164) (Kouno et al. 1995), using hexanol as fumigant. The difference in P concentration between fumigated and non-fumigated sample was considered to be microbial P. Five mL of the extract from the $NaHCO_3$ and NaOH fraction was acidified to pH 1.5 to precipitate organic matter, centrifuged and the supernatant was analysed for inorganic P. Total P in the 0.5 M $NaHCO_3$, 0.1 M NaOH and concentrated HCl fractions was determined by digestion of the extract with potassium persulfate (adjusted pH ≈ neutral) for 16 h at 90°C (Huang and Zhang 2009). Organic P (Po) in these pools is the difference between total P and inorganic P (Pi). Organic P in the 1 M HCl fraction was not determined as preliminary tests showed that the amount of organic P in this pool is very low,

which is in agreement with Tiessen and Moir (1993). Residual P in the remaining soil and soil total P of un-fractionated soil were determined by digestion with nitric and perchloric acid mixture (6:1) for 4 h. The Pi concentration in all extracts was determined using the molybdenum blue method (Murphy and Riley 1962). Resin P, bicarbonate-extractable ($NaHCO_3$) Pi and Po are considered to be labile pools (Tiessen and Moir 1993; Linquist et al. 1997), whereas hydroxide-extractable (NaOH) Pi and Po, acid-extractable (1 M and concentrated HCl) Pi and Po and residual P are less labile pools (Hedley et al. 1982; Tiessen and Moir 1993). To determine the effect of P addition on P pools prior to phase 1, the soil was sampled 24 h after P addition.

Statistical analysis

The data were analysed by analysis of variance (ANOVA); post-hoc analyses were performed using Tukey's multiple comparison test at 95% confidence intervals (GenStat® for Windows 10.0 VSN Int. Ltd, UK).

Results

Plant growth and P uptake in Phase 1 of the rotation (wheat and legume pre-crops)

At harvest, nodulation was observed in all legumes; cluster roots were formed in white lupin, but not in other legumes. There were no significant differences in shoot dry weight, but it tended to be highest in yellow lupin (Table 1). Faba bean had the highest root dry weight and wheat the lowest. Shoot P concentrations were higher in faba bean and wheat than in the two lupins. Total P uptake was greatest in faba bean and lowest in wheat. Shoot N concentration of legumes was 10 to 20-fold greater than in wheat whereas root N concentrations were 3 to 4-fold higher.

Soil pH and size of rhizosphere P pools in Phase 1

At harvest, soil pH was significantly lower than the initial pH (pH 5.4) with the greatest decrease in the unplanted control soil (pH 4.4) and the smallest in the rhizosphere of wheat (pH 4.9) (data not shown).

The addition of soluble P significantly increased the concentration of the labile inorganic P pools particularly resin P and $NaHCO_3$-Pi. Among the less labile pools, only the concentration of NaOH-Pi was significantly increased (data not shown). At harvest, irrespective of the plant species, inorganic and organic pools each accounted for 33-44% of total P. Residual P ranged from 20 to 23% and microbial P was the smallest pool representing 1 to 3% of total P (Table 2).

Microbial P was significantly higher in the rhizosphere of white lupin than in the unplanted soil and the rhizosphere of faba bean (Table 2). Compared to the unplanted soil, resin P was significantly lower in the rhizosphere of all four crops, with the strongest decrease in white lupin and smallest in wheat. All legumes depleted the $NaHCO_3$-Pi and NaOH-Pi pools compared to the unplanted soil, however there was no apparent depletion of these pools by wheat. The concentration of $NaHCO_3$-Po was significantly higher in the rhizosphere of white lupin. No significant depletion of the NaOH-Po pool was found among the crops. The concentration of the 1 M HCl-Pi pool was decreased only in the rhizosphere of yellow lupin. The concentrations of concentrated HCl-Pi and Po were significantly highest in the rhizosphere of wheat and lowest in the rhizosphere of faba bean and white lupin. There was no significant difference in residual P concentrations between rhizosphere and the unplanted soil but among the rhizosphere soils, the residual P concentration was significantly lower in white lupin than in wheat. The concentration of total P, which was determined separately; was lower in the rhizosphere of the lupin species than in the unplanted control and the wheat rhizosphere. The fractionation scheme recovered 98 to 105% of the total P from the soil (Table 2).

Plant growth and P uptake in Phase 2 of the rotation (wheat)

Shoot and root dry weights of the following wheat were highest in the previously unplanted soil (Figure 1). Without residue addition, wheat shoot and root dry weights were higher in wheat grown in the wheat pre-crop soil than in wheat grown in the legume pre-crop soils. The addition of residues to the soils pre-cropped with white lupin, faba bean and wheat decreased shoot and root dry weights of the following wheat whereas addition of yellow lupin residues had no effect.

Shoot P concentration of wheat grown in the pre-crop soils did not differ from that of wheat growing in the previously unplanted soil. Among the pre-cropped soils, shoot P concentration was highest in wheat grown in the faba bean pre-crop soil with residues and lowest in wheat grown in white lupin pre-crop soil with residues. Wheat grown in wheat pre-crop without residues had the highest root P concentration (Figure 2).

In accordance with the dry weight, P uptake of wheat grown in the previously unplanted soil was greater than that in the other treatments (Figure 3). Among the pre-crop soils without residues, P uptake of wheat grown in the soil pre-cropped with wheat was significantly higher than in the soils previously cropped with legumes. Addition of residues decreased P uptake except in the soil pre-cropped with yellow lupin.

Generally, shoot N concentration was higher in wheat grown in the pre-crop soils with residues than without residues and the previously unplanted soil (Figure 4a). Legume residue addition significantly increased the concentration of inorganic N in the bulk soil (Figure 4b).

Size and changes of P pools in the rhizosphere of wheat in Phase 2

Prior to planting of wheat, the concentration of microbial P was similar in all soils whereas the concentrations of the labile P pools (resin P and $NaHCO_3$-Pi and Po) and of the less labile pools (NaOH-Pi and residual P) were greatest in the previously unplanted soil. The concentrations of NaOH-Po, 1 M HCl-Pi and concentrated HCl-Pi were highest in the soil

previously cropped with wheat. The concentration of most P pools was lowest in the soils previously cropped with faba bean and white lupin (data not shown).

Six weeks after planting of wheat, the concentration of microbial P was higher in the rhizosphere of wheat grown in the pre-crop soils with residues than in the previously unplanted control soil or the pre-crop soils without residues (Figure 5a). The resin P, $NaHCO_3$-Pi and NaOH-Pi concentrations were highest in the rhizosphere of wheat grown in wheat pre-crop soil with residues (Figure 5b, c, d). Generally, the concentration of 1 M HCl-Pi and of concentrated HCl-Pi were higher in the rhizosphere of wheat grown in pre-crop soil with residues than without residues (Figure 5e, f). The addition of pre-crop residues significantly increased the concentration of organic P in the $NaHCO_3$, NaOH and concentrated HCl pools (Figure 6a, b, c). The concentration of residual P in the rhizosphere of wheat grown in the wheat pre-crop soil without residue addition was significantly higher than most other treatments (Figure 6d).

In the bulk soil prior to planting, resin P and $NaHCO_3$-Pi were depleted by all pre-crops with the greatest depletion by white lupin (Table 3a). The pre-crops also depleted $NaHCO_3$-Po, NaOH-Pi as well as concentrated HCl-Po with the greatest depletion by yellow lupin. The NaOH-Po and 1 M HCl-Pi pools were depleted by legumes while the concentrations of these pools were increased by the wheat pre-crop. Residual P was depleted by most pre-crops except for yellow lupin with the strongest depletion by faba bean.

In the treatment without residue addition, relative to the concentrations prior to planting of wheat, microbial P increased while resin P and $NaHCO_3$-Pi decreased in the rhizosphere of wheat (Table 3b). The depletion of resin P and $NaHCO_3$-Pi were greater in the rhizosphere of wheat grown in the previously unplanted soil than in wheat grown in the pre-cropped soils. The concentration of $NaHCO_3$-Po increased in the rhizosphere of wheat grown in the legume pre-crop soils. The concentration of NaOH-Pi decreased in the rhizosphere of wheat grown in the previously unplanted soil and in faba bean pre-crop soil while it increased in the

rhizosphere of wheat grown in white lupin, yellow lupin and wheat pre-crop soils. The NaOH-Po concentration increased significantly in the rhizosphere of wheat grown in faba bean and yellow lupin pre-crop soils. The concentrated HCl-Pi was depleted by wheat in most treatments except in yellow lupin pre-crop soil where the concentration was increased. The organic fraction of concentrated HCl-P increased in most treatments except in the rhizosphere of wheat grown in the faba bean pre-crop soil. Compared to the soil before planting, the concentration of residual P decreased most in the rhizosphere of wheat grown in the previously unplanted soil, whereas the increase was greatest in the rhizosphere of wheat growing in faba bean pre-crop soil.

In the pre-crop soils, the sum of decreases was greater than the sum of increases with the strongest decrease by faba bean and smallest by wheat; thus there was a net decrease of P concentrations compared to the unplanted soil (Table 3a). However, the reverse was true in the rhizosphere of the following wheat grown in pre-cropped soils of three legumes where the sum of decreases was smaller than the sum of increases indicating that there was an accumulation of P during the growth of the following wheat. However this was not the case in wheat grown in the previously unplanted soil and wheat pre-crop soil (Table 3b).

Discussion

The study under non-P-limiting conditions showed that in the first phase of the rotation, white lupin was able to deplete most P pools to a greater extent than the other pre-crops although its growth and P uptake was less than that of the other two legumes. In the second phase of the rotation, wheat growth and P uptake were greater in the previously unplanted soil than that of the pre-crop soil. Residue addition stimulated the accumulation of labile and less labile organic P.

Growth, P uptake and P pools of the pre-crops

Plant P concentrations were below the adequate range for all pre-crops (Reuter and Robinson 1997). However, growth and thus P uptake of the legumes was significantly greater than of wheat. Wheat shoot N concentrations were low, suggesting that despite addition of N via the nutrient solution, wheat growth was limited by N. The result of this study is in agreement with Nuruzzaman et al. (2005b) who showed that at high P supply in an acidic soil (pH_{CaCl2} 4.9), legume growth was greater than that of wheat although P concentrations were similar. The present study also demonstrated that the ability to deplete labile and less labile P pools differed greatly among legume species, but that wheat was also able to deplete less labile P pools to some extent. White lupin depleted P pools more strongly than wheat and the other legume species with the greatest depletion in NaOH-Pi (Table 2) which is in agreement with our previous study in an alkaline soil (Mat Hassan et al. 2012b).

In this study, resin P was significantly depleted by all legumes and wheat; confirming other studies (Vu et al. 2008; Wang et al. 2011; Mat Hassan et al. 2012b) that this labile pool is the main source of plant available P. Recent studies have shown no differences in the ability to access different P pools among legumes and non-legume species despite differences in total P uptake (Nuruzzaman et al. 2006; Rose et al. 2010). In our study, however, all legumes depleted the inorganic P fractions of the $NaHCO_3$ and NaOH pools to a greater extent than wheat, suggesting that in this acidic soil, legumes were better in mobilising these pools than wheat. The differential results in these studies could be due to contrasting soil pH and dynamics of P pools as well as the use of different crop species/cultivars (Vu et al. 2010). The greater depletion of $NaHCO_3$-Pi and NaOH-Pi by the legumes is probably related to the release of carboxylates, which has been shown to be greater in legumes than in wheat (Nuruzzaman et al. 2005b; Rose et al. 2010). Carboxylates exuded in the rhizosphere can release P from Al- and Fe-oxide via ligand exchange (Gerke 1992; Jones 1998).

Among the legumes, white lupin induced the greatest depletion of the less labile pools, suggesting that white lupin is particularly efficient in mobilising P. In agreement with this depletion in the acidic soil used here, white lupin also showed the greatest depletion of less labile P pools in an alkaline soil (Mat Hassan et al. 2012b). This result can be explained by the cluster roots which have been shown to release large amounts of carboxylates (Neumann and Römheld 1999; Neumann et al. 2000; Li and Liang 2005; Shen et al. 2005). However, despite the strong depletion of less labile pools by white lupin, its P uptake was lower than in faba bean and yellow lupin. Thus, although the inorganic P pools were depleted in the rhizosphere of white lupin, the majority of the mobilised P did not appear to be taken up by the roots. This lack of uptake of mobilised inorganic P could be due to (i) diffusion of the mobilised P from the rhizosphere into the bulk soil, (ii) the small root system of white lupin not being able to retrieve the mobilised P, and (iii) transformation into organic P. The latter is indicated by the fact that although the organic P fractions were also depleted in the rhizosphere of white lupin, the extent of depletion was less than in the other two legumes.

Growth of the following wheat

In agreement with our previous study in an alkaline soil (Mat Hassan et al. 2012a) but in contrast to many other studies (Horst et al. 2001; Kamh et al. 2002; Nuruzzaman et al. 2005a; Jemo et al. 2006), legume pre-crops did not improve growth of the following wheat compared to wheat after wheat and all pre-crops reduced wheat growth compared to wheat growing in previously unplanted soil which is consistent with Nuruzzaman et al. (2005b) in an acidic soil (pH_{water}5.3). The highest wheat growth in the previously unplanted soil can be explained by the higher nutrient availability as nutrients added at the start of phase 1 would still be present in the previously unplanted soil whereas they were taken up by the pre-crops. The negative effect of the pre-crops on the growth of the following wheat may be related to the low shoot P and N concentrations which indicate deficiency although nutrients were added to wheat in the second phase.

The growth of wheat in the wheat pre-crop soil was greater than in legume pre-crop soils (Figure 1) probably due to the higher P availability since the wheat pre-crop depleted resin P, $NaHCO_3$-Pi and NaOH-Pi to a lesser extent than the legumes (Table 2). This result is in contrast to the study by Nuruzzaman et al. (2005b) where the growth of wheat grown in legume pre-crop soils was greater than in wheat pre-crop. This contrasting result could be due to the addition of soluble P at the start of the pre-crop phase in the present study which was 5 times greater than the rate used by Nuruzzaman et al. (2005b). The results suggest that, in the soil used here, the added soluble P had remained available throughout the experiment and hence increased the growth of the following wheat.

In the present study, the addition of the residues of the pre-crops decreased the growth of wheat. Recently, Hasbullah (2011) also reported negative effects of addition of legume residues on the growth of wheat in an alkaline soil at lower P supply (15 mg P kg^{-1}). Available N and labile inorganic P concentration in the soil at the harvest of wheat were higher in soil with residues, however, the negative effect of the residues on wheat growth may be due to temporary immobilisation of N and P in the first weeks of wheat growth. At the time of harvest, residue addition increased wheat shoot N concentrations suggesting that wheat had not had sufficient time to respond to the increased N uptake by enhancing shoot growth. A further explanation of the poorer growth of wheat in soil with residues is the release of toxic compounds from the decomposing residues may also contribute to a reduction in wheat growth. Although residue addition to pre-crop soils decreased the growth of the following wheat, the residues of yellow lupin had the least detrimental effect which could be due to high N concentration in the residues (Table 1). High N concentration in the added residue results in greater decomposition rate (Cheshire and Chapman 1996), thus releasing P and other nutrients for uptake by the following wheat.

Changes in rhizosphere P pools of the following wheat

Without residue addition, irrespective of the preceding crops, depletion in the rhizosphere of the following wheat was greatest in the labile pools resin P and $NaHCO_3$-Pi, suggesting that these pools served as the principal sources of P for wheat (Table 3b). This is in agreement with observations by Vu et al. (2008) and our own study in an alkaline soil at high P availability (Mat Hassan et al. 2012a).

Compared to wheat grown in previously unplanted soil, the labile inorganic P pools (resin and $NaHCO_3$-Pi) were less depleted in the rhizosphere of wheat grown in pre-crop soil. Further, microbial P, $NaHCO_3$-Po, NaOH-Pi and residual P accumulated in the rhizosphere of wheat grown in pre-crop soil. The smaller depletion or accumulation of various P pools in wheat after pre-crops compared to wheat in previously unplanted soil can be explained by its poorer growth and thus P uptake. However this also indicates that more P would be available to wheat in the later stages of growth which could be of advantage during flowering or grain-filling.

The increase in the NaOH-Po concentration suggests that synthesis of organic P by soil microbes was particularly stimulated in the rhizosphere of wheat growing in the soils previously cropped with faba bean and yellow lupin (Table 3b).

The net increase in P pool concentration (the sum of increases minus the sum of decreases) in the rhizosphere of wheat can be explained by the depletion of available P in the rhizosphere resulting in P diffusion from the bulk soil to the rhizosphere soil to regain equilibrium which might be enhanced by the diffusion of carboxylates and phosphatases into the bulk soil. Diffusion of P from the bulk into the rhizosphere will also have occurred in the pre-crop phase, but did not result in accumulation due to the greater P uptake in this phase

The concentration of most P pools and particularly of the organic P pools was higher in the rhizosphere of wheat growing in the pre-crop soils with residues than without residues. The

higher concentration of most P pools in the rhizosphere of wheat growing in the pre-crop soils with residues than without residues can be explained by (i) P release from the decomposing residues, (ii) reduced P uptake because of the poorer growth of the wheat, and (iii) stimulation of microbial activity and thus synthesis of organic P (in the microbial biomass) as well as P mobilisation by microbial carboxylates and phosphatases. Soil microbes are often limited by C, therefore addition of C increases microbial growth and nutrient uptake (De Nobili et al. 2001; Hoyle et al. 2008). In the present study this is evident in the greater concentration of microbial P in the rhizosphere of wheat grown in the pre-crop soils with residues than without residue addition (Figure 5a). A proportion of the C in plant residues is easily available and therefore stimulates microbial growth and P immobilisation which can limit the plant growth (McLaughlin and Alston 1986). However, over time as the C availability decreases, microbial P represents a slow and constant source of available P through decomposition of dead microbial cells (Oberson et al. 2001; Marschner et al. 2011).

Conclusions

This study confirmed the greater ability of white lupin to mobilise labile and less labile P pools compared to other legumes and wheat. However, all legumes and also wheat were able to deplete P pools that are considered to be less labile, suggesting the capacity to utilise P from the so-called soil P bank. Therefore, the greater growth and P uptake of the legumes compared to wheat appears to be due to other factors, for example stronger root growth and thus exploitation of the soil particularly in the early stages of growth contribute to the higher P uptake of the legumes. The accumulation of organic P in the rhizosphere of wheat particularly in legume pre-crop soils warrants further studies to determine if this organic P becomes available over time. Further, this suggests that increased phosphatase activity in the rhizosphere could be a selection criterion for developing P-efficient cereal cultivars.

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We are grateful for financial support from the Australian Research Council, Ministry of Higher Education Malaysia and Universiti Sains Malaysia.

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Table 1: Plant dry weight, P concentration and uptake, N concentration and uptake, and C concentration of wheat (at grain filling) and three grain legumes (at pod set) grown in a sandy loam soil (n=4). Values in the same column followed by different letters are significantly different ($P \leq 0.05$) by Tukey's multiple comparison tests.

Plants species	Dry weight ($g\ pot^{-1}$)		P concentration ($mg\ g^{-1}$)		N concentration ($mg\ g^{-1}$)		C concentration ($mg\ g^{-1}$)		P uptake ($mg\ pot^{-1}$)	N uptake ($mg\ pot^{-1}$)
	shoot	root	shoot	root	shoot	root	shoot	root		
Faba bean	21.8 b	15.9 c	2.02 c	1.54 a	20.5	21.5	403	317	72.9 d	696.7 d
White lupin	20.6 b	7.5 b	1.74 ab	1.65 a	15.5	25.4	428	391	47.7 b	491.0 b
Yellow lupin	22.0 b	6.2 b	1.51 a	1.72 a	30.1	22.7	398	375	52.7 c	651.5 c
Wheat	13.6 a	3.5 a	1.84 bc	1.66 a	1.5	5.6	391	353	38.5 a	34.7 a

Table 2: Concentrations of different P pools and total P in soil of the unplanted control and the rhizosphere of pre-crops faba bean, white lupin, yellow lupin and wheat at harvest (n=4). Values in the same column followed by different letters are significantly different ($P \leq 0.05$) by Tukey's multiple comparison tests.

Plant species	Microbial P	Resin P	$NaHCO_3$		NaOH		HCl-Pi	Conc. HCl		Residual P	Total P	Recovery (%)*
			Pi	Po	Pi	Po		Pi	Po			
					mg P kg^{-1} soil							
Control	8.1a	38.8 c	46.7 b	64.6 abc	114.5 c	114.0 a	8.6 b	36.5 b	20.3 b	109.2 ab	531.4 b	104
Faba bean	9.2 a	25.3 ab	29.4 a	70.5 bcd	91.4 b	113.9 a	8.8 b	27.4 a	15.5 a	100.8 ab	496.8 ab	99
White lupin	12.1 b	19.0 a	22.2 a	79.4 d	76.9 a	106.5 a	8.2 ab	25.9 a	13.4 a	97.0 a	466.9 a	98
Yellow lupin	10.5 ab	22.5 a	29.9 a	59.0 ab	89.5 ab	103.5 a	5.9 a	36.4 b	20.1 b	106.0 ab	467.9 a	103
Wheat	10.7 ab	29.7 b	41.4 b	56.9 a	111.5 c	114.4 a	8.7 b	39.3 b	21.0 b	110.4 b	527.4 b	103

*Percent recovery is the sum of all P fractions in percentage of total P measured in the un-fractionated soil.

Table 3: (A) Changes in P pools of the bulk soil relative to the unplanted control soil induced by pre-crops prior to planting of wheat and (B) changes in P pools in the rhizosphere soil of following wheat grown for 6 weeks in the unplanted control soil and soils pre-cropped with faba bean, chickpea, white lupin and wheat relative to those in the bulk soil of each pre-crop prior to planting (n=4). Negative values indicate decreases while positive values increases. *indicates significant difference compared to the control soil (section A), while values in the same column followed by the same letter are not significantly different (section B) ($P \leq 0.05$) by Tukey's multiple comparison tests.

	Changes in P pools (mg P kg^{-1})											
	Microbial P	Resin P	$NaHCO_3$		NaOH		HCl-Pi	Conc. HCl		Residual P	Sum of decreases	Sum of increases
			Pi	Po	Pi	Po		Pi	Po			
Bulk soil	A: Changes by pre-crops											
Faba bean	-0.3	-13.4*	-16.0*	-10.5	-27.7*	-7.7	-1.3	-2.9	-0.4	-16.2*	-96.4	0
White lupin	0.7	-15.1*	-16.7*	-7.0	-27.9*	-1.6	-1.4	0	-3.1	-8.7	-81.5	0.7
Yellow lupin	-0.8	-4.6	-8.7	-12.4*	-33.4*	-1.8	-1.6	-9.3*	-2.8	1.7	-75.4	1.7
Wheat	0.8	-8.0	-9.2	-0.2	-15.8	4.0	0.4	4.6	-4.9*	-3.2	-41.3	9.8
Rhizosphere soil	B: Changes by the following wheat											
W-Control	4.9 a	-19.9 a	-14.8 a	-8.9 a	-22.3 a	0.4 a	-1.1 a	-7.9 a	3.3 ab	-10.1 a	-85.2	8.6
W-Faba bean	6.5 a	-9.8 c	-9.4 b	6.7 b	-6.3 b	14.2 b	-1.6 a	-0.7 bc	-0.4 a	9.0 d	-28.2	36.4
W-White lupin	6.3 a	-5.4 e	-6.7 bc	6.0 b	11.7 c	-1.1 a	-0.6 a	-5.4 ab	6.5 b	-1.4 bc	-20.6	30.5
W-Yellow lupin	7.4 a	-12.9 b	-9.7 b	9.0 b	9.8 c	14.4 b	0.1 a	5.3 c	2.5 ab	-7.0 ab	-19.9	48.5
W-Wheat	9.2 a	-7.9 d	-4.8 c	-3.6 a	4.4 c	-0.2 a	-1.2 a	-10.0 a	3.8 ab	6.4 cd	-27.7	23.8

[A] Difference in concentration of a given P pool between the bulk soil of legume pre-crops and the unplanted control soil

[B] Difference in concentration of a given P pool between the rhizosphere soil of wheat grown in legume pre-crop soil and the concentration prior to planting of the wheat

List of figures

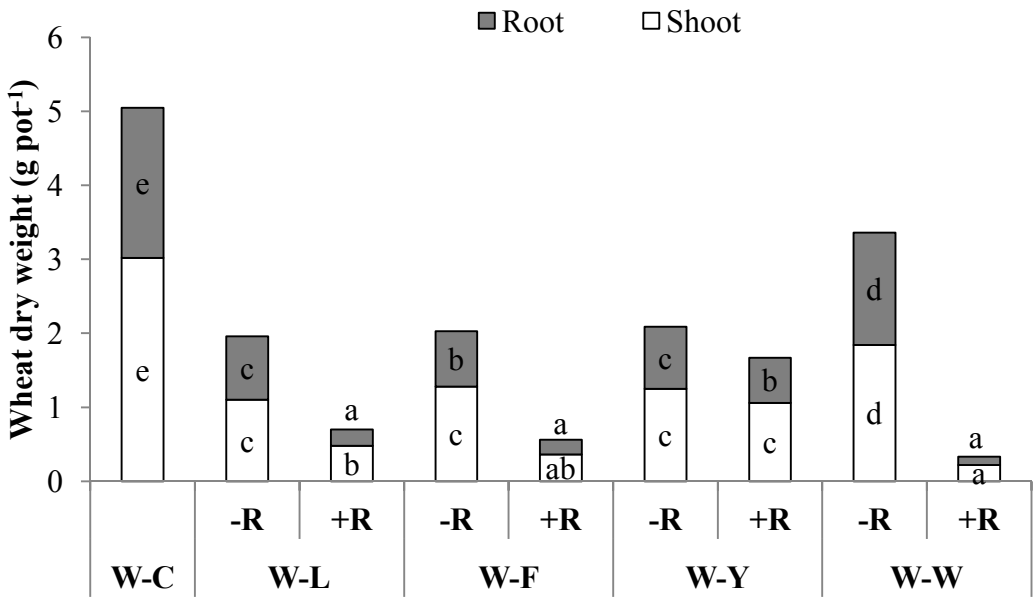


Figure 1

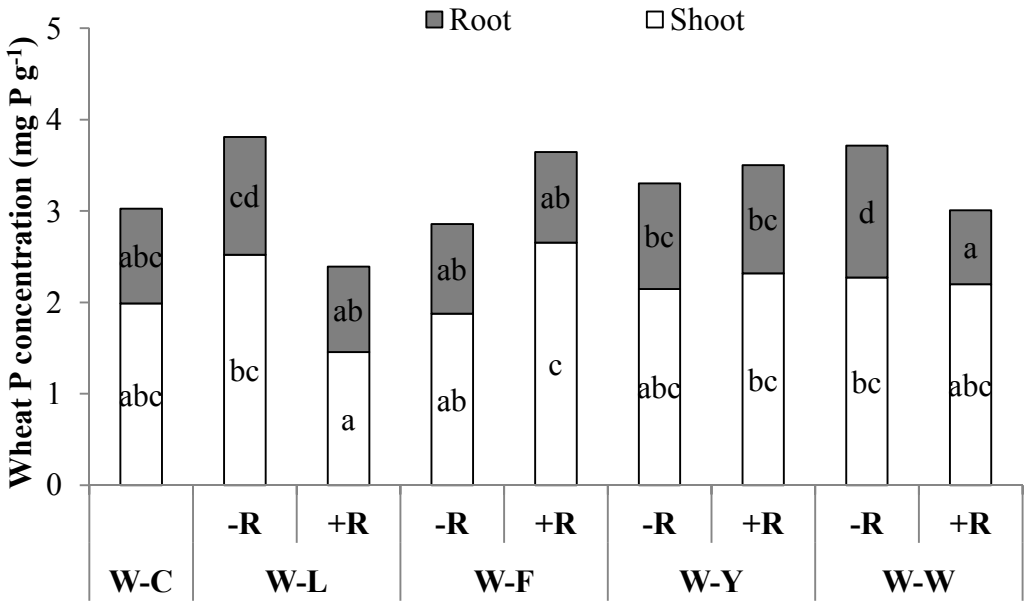


Figure 2

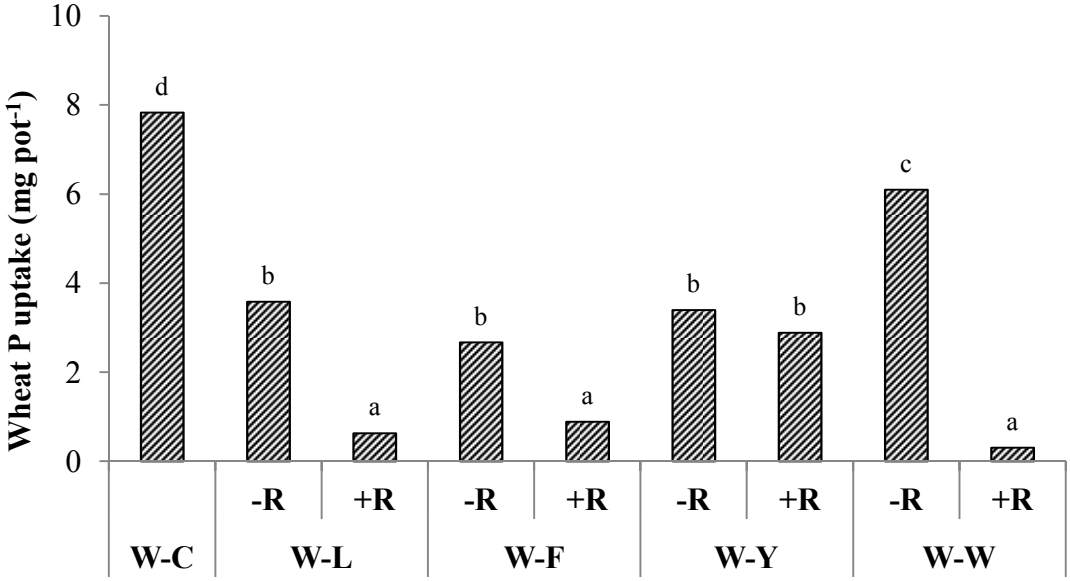
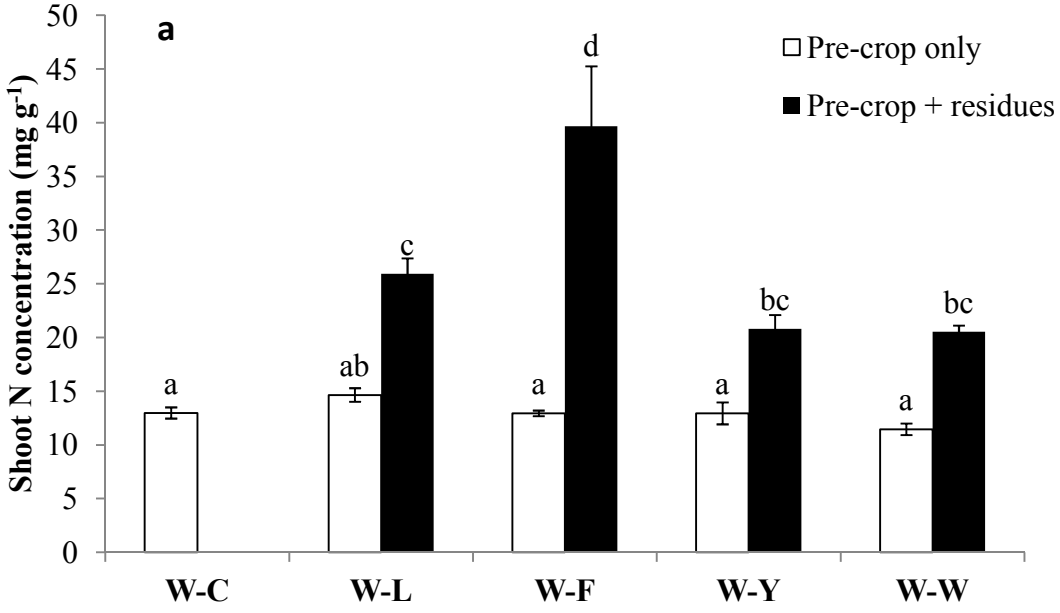

Wheat P uptake (mg pot-1)
10
8
6
4
2
0
d
b
a
b
a
b
b
c
a
-R
+R
-R
+R
-R
+R
-R
+R
W-C
W-L
W-F
W-Y
W-W

Figure 3


a
Shoot N concentration (mg g-1)
50
45
40
35
30
25
20
15
10
5
0
Pre-crop only
Pre-crop + residues
a
ab
c
a
d
a
bc
a
bc
W-C
W-L
W-F
W-Y
W-W

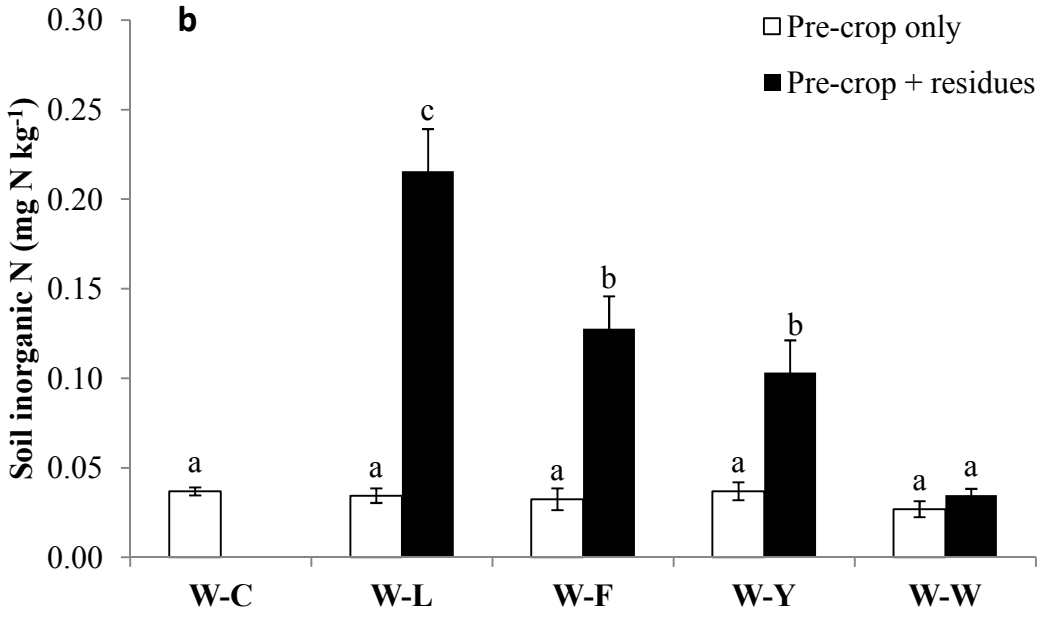

b
Pre-crop only
Pre-crop + residues
Soil inorganic N (mg N kg-1)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
a
c
a
b
a
b
a
a
a
W-C
W-L
W-F
W-Y
W-W

Figure 4

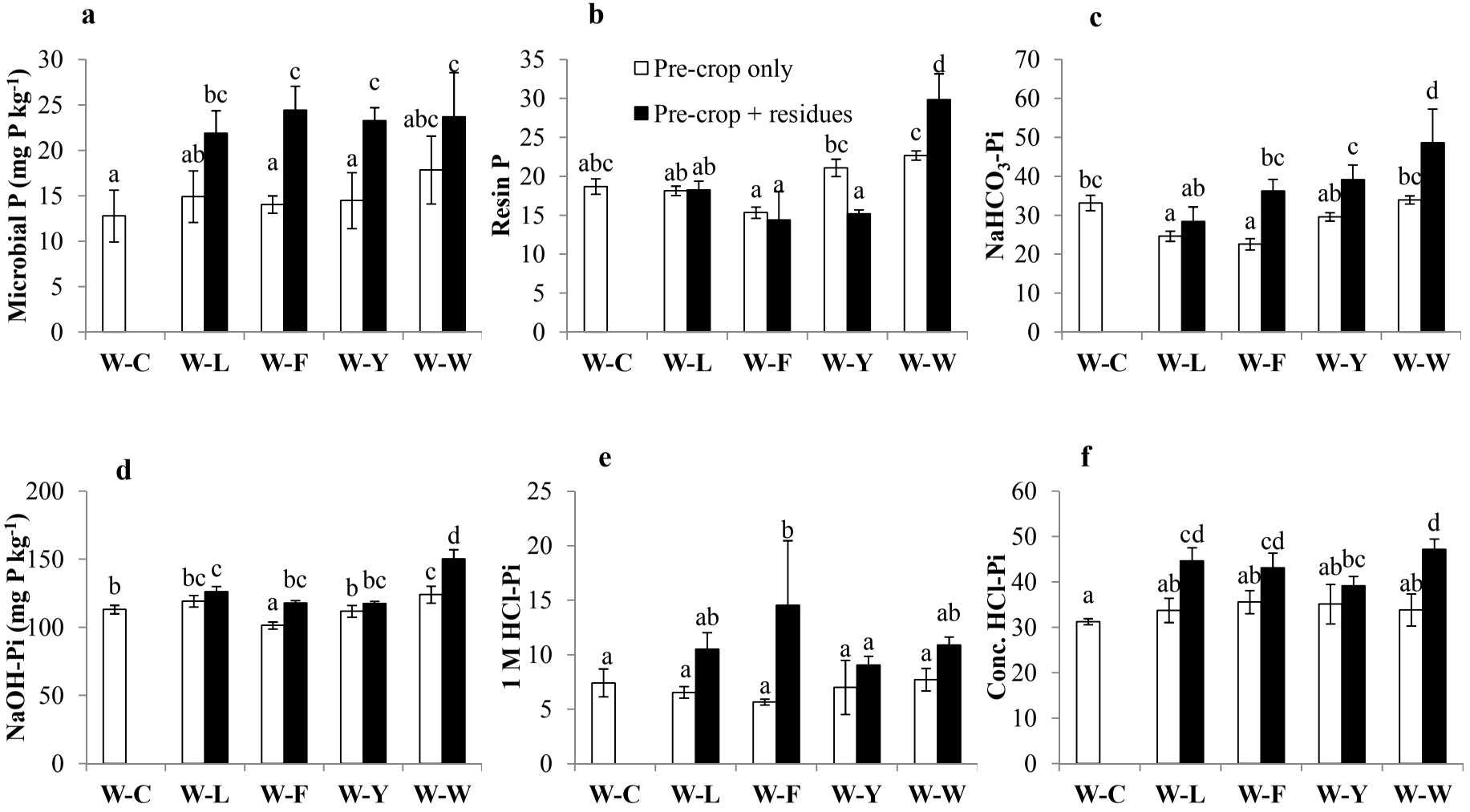

a
Microbial P (mg P kg-1)
W-C
W-L
W-F
W-Y
W-W
b
Resin P
Pre-crop only
Pre-crop + residues
c
NaHCO3-Pi
d
NaOH-Pi (mg P kg-1)
e
1 M HCl-Pi
f
Conc. HCl-Pi

Figure 5

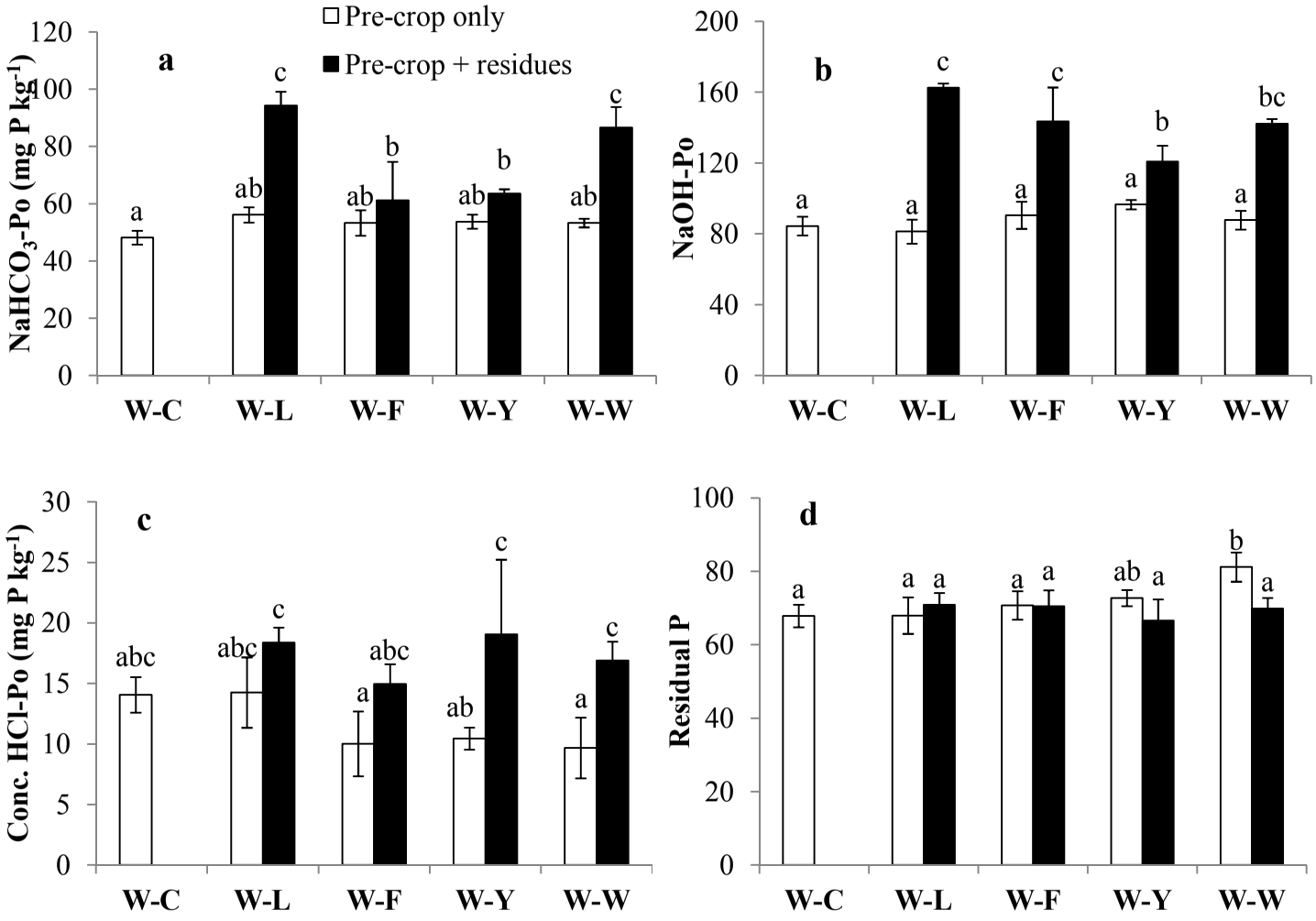

Pre-crop only
Pre-crop + residues
a
b
c
d
NaHCO3-Po (mg P kg-1)
NaOH-Po
Conc. HCl-Po (mg P kg-1)
Residual P
W-C
W-L
W-F
W-Y
W-W

Figure 6

Chapter 5

Growth and rhizosphere P pools of legume-wheat rotations at low P supply

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STATEMENT OF AUTHORSHIP

Growth and rhizosphere P pools of legume-wheat rotations at

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Biology and Fertility of Soils; published paper

Mat Hassan, H (Candidate)

Performed analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author.

I hereby certify that the statement of contribution is accurate.

Signed Date

Hasbullah

Contributed to planning of experiment, performed analysis on samples and interpreted data

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed Date

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Supervised development of work, data interpretation and manuscript evaluation.

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Growth and rhizosphere P pools of legume-wheat rotations at low P supply

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Abstract

Legume pre-crops may increase P uptake of the following wheat but the mechanisms behind this effect are unclear. A rotation study was carried out to assess the concentrations of rhizosphere P pools of three grain legumes and wheat (phase 1) and their effects on P uptake and P pools in the rhizosphere of the following wheat (phase 2). Faba bean, chickpea, white lupin and wheat were grown for 10 weeks in a loamy sand soil with low P availability. The following wheat was grown in the pre-crop soil with and without addition of pre-crop residues. Among the pre-crops, white lupin had the strongest effect on the P pools, it depleted the labile P pools; resin P and $NaHCO_3$-Pi and also the less labile P pools; NaOH-Pi and residual P, whereas the concentration of $NaHCO_3$-Po was higher than in the rhizosphere of the other pre-crops. White lupin had a smaller biomass compared to faba bean which depleted the P pools to a lesser extent. Phosphorus uptake of the following wheat was greatest in white lupin pre-crop soil. Chickpea increased P uptake of the following wheat when residues were added. In presence of residues, wheat after legumes depleted labile P pools to a greater extent than wheat after wheat but this coincided with greater P uptake only in wheat after chickpea and white lupin, which may be explained by the small root biomass of wheat after faba bean. The results show that the greater P uptake of the following wheat induced by pre-crops may be due to two mechanisms: P mobilisation (white lupin) or P addition with legume residues

(chickpea). This study further showed that P uptake by a crop is only partly a function of the depletion of P in the rhizosphere; another important factor is the ability to exploit a large soil volume.

Keywords

Crop rotation, Grain legumes, P fractionation, P uptake, Pre-crops, Wheat

Introduction

Phosphorus (P) is essential for all living microorganism because it is required for energy metabolism and biosynthesis of nucleic acid and membranes (Brady and Weil 2002). In many soil world-wide, P limits crop production due to its poor availability in the soil. In order to overcome P deficiency and increase crop yield, water-soluble P fertiliser is frequently applied by farmers. However, plants typically take up only 45% of this added P (Mózner et al. 2012) whereas the remaining P is converted to less available forms due to various chemical reactions in the soil which vary with soil pH (Sanyal and De Datta 1991). The accumulation of P as a result of P fertilisation has lead to a build up of a soil P bank or reserve P which some crops such as legumes are able to access (Vu et al. 2008; Vu et al. 2010).

Legumes are increasingly used in intercropping as well as crop rotations due to their beneficial effect on soil N and P availability. Further, previous studies have shown that some legumes are able to mobilise P from soils with both low and high P availability (Kamh et al. 1999; 2002; Nuruzzaman et al. 2006; Rose et al. 2010; Wang et al. 2011). The ability of legumes to mobilise P is due to : (i) modification of rhizosphere physico-chemical properties by root activity e.g. pH changes due to acidification or alkalisation (Hinsinger 1998; Morel and Hinsinger 1999; Hinsinger et al. 2003), (ii) exudation of carboxylates (organic acid anions) (Veneklaas et al. 2003), and (iii) root or microbially derived phosphatases which mineralise soil organic pools (Nuruzzaman et al. 2006; Nannipieri et al. 2011).

Further, several studies reported positive effects on growth and P uptake of cereals following legumes at low P supply even with adequate N supply which are thought to be due to (i) increased soil P availability from P mobilisation by legumes (Kamh et al. 2002) (ii) P addition with legume residues (Horst et al. 2001; Nuruzzaman et al. 2005a), and (iii) P mobilisation during decomposition of residues (Kamh et al. 2002). However, our previous studies on both acid and alkaline soils found that when sufficient P was supplied to the legume pre-crops, they had a negative effect on growth and P uptake on the following wheat both in absence or presence of residues (Mat Hassan et al. 2011a). In order to investigate if the effect of the legumes on the following wheat is different at low P availability, a study was carried out with the same soil as in one of our previous experiments (alkaline loamy sand) with low P supply. The aims of this study were to investigate (i) the concentration of rhizosphere P pools in three grain legume and wheat pre-crops (phase 1 of the rotation) and (ii) the effect of the pre-crops in presence or absence of their residues on growth, P uptake and rhizosphere P pools of the following wheat (phase 2 of the rotation).

Materials and Methods

A loamy sand soil with low P availability from natural vegetation (0 - 10 cm) was collected in Monarto, South Australia (latitude 35° 05´ S, longitude 139° 04´ E, elevation 212 m). The soil was air-dried and passed through a 2-mm sieve. The properties of the soil are as described in the previous study (Mat Hassan et al. 2012b).

Experimental design

The crop rotation experiment was carried out in a glasshouse with natural light from May to July (autumn to winter in the southern hemisphere); the temperature ranged from 15 to 30 ºC. The pots of both phases were completely randomised. The soil was mixed thoroughly with 15 mg P kg^{-1} as KH_2PO_4 and placed in 2 kg sealed pots. In the first phase of the rotation, four pre-germinated seeds of wheat (*Triticum aestivum* L. cv. Krichauff) and 3 grain legumes: faba

bean (*Vicia faba* L. cv. Fiesta), chickpea (*Cicer arietinum* L. cv. Kabuli) and white lupin (*Lupinus albus* L. cv. Luxor) were sown at 2 cm depth. Each legume was inoculated with respective rhizobia strain: *Bradyrhizobium* strain WU425 (group G) for white lupin; *Rhizobium* strain WSM1455 (group F) for faba bean; *Rhizobium* strain CC1192 (group N) for chickpea while wheat was supplied with 85 mg N kg^{-1} or 170 mg pot^{-1} as NH_4NO_3. The plants were thinned to two plants per pot after two weeks. Nutrient solution (except P and N), as described in Nuruzzaman et al. (2005b), was added at 40 mL per pot and per week to ensure pre-crop growth was not limited by these nutrients. The composition of nutrient solution was (in micromolars): $CaCl_2$, 150; K_2SO_4, 100; $MgSO_4$, 54; H_3BO_3, 2.4; $MnSO_4$, 0.24; $ZnSO_4$, 0.1; Na_2MoO_4, 0.03; $CuSO_4$, 0.02; $CoCl_2.6H_2O$, 0.001. Unplanted soil representing bulk soil (control) was treated in the same manner. The soil was maintained at 70% water holding capacity (WHC) throughout the experiment by weight using reverse osmosis water (RO). There were four replicate pots per plant species and the unplanted control.

After 10 weeks, rhizosphere soil was sampled from each plant species. Plant shoots and roots were harvested and oven dried (80°C for 48 h) for further analyses and used in the second phase of the rotation. Plant materials were cut to 1-2 cm and thoroughly mixed with the pre-crop soil prior to planting of following wheat. Four pre-germinated wheat seeds (cv Krichauff) were grown in previously unplanted soil, pre-crop soil (soil previously cropped with wheat and legumes) without residue addition and pre-crop soils with a mixture of shoot and root residues (2.4 g shoot kg^{-1} + 0.4 g root kg^{-1}). The amount of P added with the residues was: wheat, 6.7; faba bean, 4.8; chickpea, 3.8 and white lupin, 6.4 mg P kg^{-1}. The treatments (n=4) were as follows: wheat grown in the previously unplanted soil, wheat pre-crop soil with and without residues, faba bean pre-crop soil with and without residues, chickpea pre-crop soil with and without residues and white lupin pre-crop soil with and without residues. The pots were watered with RO water to 70% WHC throughout the experiment by weight but no nutrient solution was added. Wheat plants were harvested 6 weeks after sowing.

Plant and soil analyses

At the end of both phases, the shoots were cut at the soil surface, the roots were gently removed from the pots and soil loosely adhering to the roots was shaken off. The rhizosphere soil, the remaining tightly adhering soil, was collected by gentle brushing, and stored at -20°C for analysis of soil total P and P fractionation. Despite extra care taken during the sampling, there was some root breakage and could be included in the samples. The shoots and roots were rinsed in RO water before drying and oven-dried at 80ºC for 48 h. Then, the dried materials were ground separately and digested in nitric and perchloric acid mixture (6:1) for 5 h for measurement of plant total P using the vanado-molybdate method (Hanson 1950). For determination of soil P pools, sequential fractionation was carried out using the method described by Tiessen and Moir (1993) with slight modifications. Fractionation removes the most labile P followed by more stable or less labile P forms by using mild to stronger extractants sequentially (Tiessen and Moir 1993). Briefly, 1 g of rhizosphere and bulk soil (<0.5 mm) was extracted sequentially with 30 mL of the following solutions: 1) 0.5 M $NaHCO_3$; 2) 0.1 M NaOH; 3) 1 M HCl; 4) 0.1 M NaOH and 5) concentrated HCl. Resin P and microbial P, which were not included in the sequential fractionation, were determined simultaneously in a separate aliquot of soil using anion-exchange resin membranes (BDH #55164) (Kouno et al. 1995), using hexanol as fumigant instead of chloroform (McLaughlin et al. 1986). The resins were eluted with 0.1 M NaCl/HCl and P concentration in the extracts was determined after Murphy and Riley (1962). The difference in P concentration between hexanol-fumigated and non-fumigated sample was considered to be microbial P. Total P in the 0.5 M $NaHCO_3$, 0.1 M NaOH and concentrated HCl fractions was determined by digestion of each extract with potassium persulfate (adjusted pH ≈ neutral) for 16 h at 90°C (Huang and Zhang 2009). Organic P (Po) in these pools is the difference between total P and inorganic P (Pi). Residual P in the remaining soil and soil total P of un-fractionated soil were determined by digestion with nitric and perchloric acid mixture (6:1) for 4 h (Kuo 1996). The

Pi concentration in all extracts was determined using the molybdenum blue method (Murphy and Riley 1962). Resin P, bicarbonate-extractable ($NaHCO_3$) Pi and Po are considered to be labile pools (Hedley et al. 1982; Tiessen and Moir 1993), whereas hydroxide-extractable (NaOH) Pi and Po, acid-extractable (1 M and concentrated HCl) Pi and Po and residual P are less labile pools (Hedley et al. 1982; Tiessen and Moir 1993).

Statistical analysis

Data were analysed by analysis of variance (ANOVA) using Genstat 11th edition (GenStat® for Windows 10.0 VSN Int. Ltd, UK). Means were tested using Tukey's at 95% confidence intervals. Significance differences refer to $P \leq 0.05$.

Results

Plant growth and P uptake in wheat and legume pre-crops

Shoot and root dry weights were significantly greater in faba bean and chickpea than in wheat and white lupin. Shoot P concentration was greatest in wheat and lowest in white lupin and the reverse was true for root P concentration. Shoot N concentrations of legumes were 1.6 to 2-fold greater than in wheat while root N concentrations were 2.3 to 2.8-fold higher. Among the pre-crops, faba bean had the highest P uptake while it was lowest in white lupin (Table 1).

Size of P pools in the rhizosphere of wheat and legume pre-crops

At harvest, irrespective of the pre-crop species, microbial P was the smallest pool representing 2 to 5% of total P. Inorganic pools accounted for 51 to 58% of total P while organic and residual P pools each accounted for 18 to 25% of total P (Figure 1a-e).

Compared to the unplanted soil, the microbial P concentration was significantly higher only in the rhizosphere of white lupin (Figure 1a). The resin P concentration was significantly lower in the rhizosphere of all pre-crops than in the unplanted soil, with the largest decrease in white lupin where the concentration was significantly lower than in wheat (Figure 1a).

Compared to the unplanted soil, the concentration of $NaHCO_3$-Pi was significantly lower in the rhizosphere of all pre-crops with a greater decrease in the rhizosphere of legumes than in wheat. The concentration of $NaHCO_3$-Po did not differ between the unplanted soil and most rhizosphere soils except for white lupin where the concentration was significantly higher than in the other pre-crops (Figure 1b). Compared to the unplanted soil, the concentration of NaOH-Pi was significantly lower only in the rhizosphere of white lupin. The concentration of NaOH-Po was significantly lower in the rhizosphere of white lupin than the other pre-crops and the unplanted soil (Figure 1c). There was no significant difference in the concentration of concentrated HCl-Pi among the rhizosphere soil of all pre-crops but it was significantly lower in the rhizosphere of chickpea than the unplanted soil. The concentration of concentrated HCl-Po also did not differ between the unplanted soil and rhizosphere soil but it was significantly lower in the rhizosphere of white lupin than that in faba bean and chickpea (Figure 1d). The concentration of 1 M HCl-Pi did not differ between the unplanted soil and rhizosphere soil. Compared to the unplanted soil, the concentration of residual P was significantly lower in the rhizosphere of wheat and white lupin (Figure 1e).

Growth and P uptake of the following wheat

Shoot and root dry weight were greatest in wheat grown in the previously unplanted soil while they were lowest in wheat grown in the soil previously cropped with faba bean with residue addition (Table 2). The addition of residues to faba bean pre-crop soil and wheat pre-crop soil decreased shoot dry weight of the following wheat by 50% and 18% whereas addition of chickpea residues increased it by 45%, compared to wheat grown in the pre-cropped soils without residues. Addition of white lupin residues to the pre-cropped soils had no effect on shoot dry weight of the following wheat.

Shoot P concentration of wheat grown in the soils pre-cropped with legumes with and without residue addition was significantly greater than in the previously unplanted soil. In the

wheat and faba bean pre-crop soils, shoot P concentration was higher in wheat grown in pre-crop soil with residues than without residue addition (Table 2).

Shoot N concentration of wheat grown in the previously unplanted soil was significantly greater than that of wheat in pre-crop soil without residue addition (Table 2). With residue addition only wheat grown in faba bean pre-crop soil had a higher shoot N concentration than wheat grown in the previously unplanted soil (Table 2).

Phosphorus uptake of wheat grown in chickpea pre-crop soil with residues was significantly higher than in wheat grown in the previously unplanted soil (Table 2).

Size of the P pools in the rhizosphere of the following wheat

After six weeks, relative to wheat grown the previously unplanted soil, the concentration of microbial P was significantly higher only in the rhizosphere of wheat grown in white lupin pre-crop soil (Figure 2a). Compared to pre-crop soil without residues, the microbial P concentration was not affected by residue addition. But compared to wheat grown in the unplanted soil, residue addition to wheat, chickpea and white lupin pre-crop soil increased microbial P (Figure 2a). Compared to wheat grown in the previously unplanted soil, the concentrations of resin P and $NaHCO_3$-Pi were significantly greater in the rhizosphere of wheat grown in wheat pre-crop soil with residue addition whereas the concentrations were lower in the rhizosphere of wheat grown in legume pre-crops with and without residue addition except for wheat grown in white lupin pre-crop soil without residue addition (Figure 2b, c). The concentration of NaOH-Pi was similar in most treatments except in the rhizosphere of wheat grown in faba bean pre-crop soil with residue addition where it was significantly lower whereas it was highest in the rhizosphere of wheat in white lupin pre-crop soil without residues (Figure 2d). Without residue addition, the concentration of 1 M HCl-Pi was significantly greater in the rhizosphere of wheat grown in the white lupin pre-crop soil compared to the previously unplanted soil and the other pre-crops (Figure 2e). Compared to

wheat grown in the previously unplanted soil, the concentration of concentrated HCl-Pi was significantly greater in the rhizosphere of wheat grown in the pre-cropped soils without residues except in the faba bean pre-crop soil (Figure 2f). Addition of residues decreased the concentration of concentrated HCl-Pi in all pre-crop soils to similar values as in the previously unplanted soil.

Compared to wheat grown in the previously unplanted soil, the concentration of organic P in the $NaHCO_3$ fraction was similar in the rhizosphere of wheat grown in the pre-cropped soils with or without residue addition (Figure 3a). Without residue addition, the concentration of NaOH-Po did not differ between wheat grown in previously unplanted soil and in pre-crop soils (Figure 3b). However addition of residues to the chickpea and white lupin pre-crop soils increased the concentration of NaOH-Po compared to wheat grown in previously unplanted soil. There was no significant difference of concentrated HCl-Po concentration between the rhizosphere soil of wheat grown in the previously unplanted soil and wheat grown in the pre-cropped soils with or without residues (except white lupin pre-crop soil where this pool was not detectable) (Figure 3c). The concentration of residual P in the rhizosphere of wheat did not differ between previously unplanted soil and pre-crop soil with or without residues, except for wheat in chickpea pre-crop soil with residues where the concentration was significantly lower (Figure 3d).

Discussion

In the pre-crop phase of this study, although its growth and P uptake was lowest, white lupin depleted most P pools to a greater extent than the other pre-crop species. In the subsequent phase, growth and P uptake of the following wheat were greater in the previously unplanted soil than in the pre-crop soils. Compared to the other pre-crops, white lupin induced greater growth and P uptake of the following wheat.

Phase 1of the rotation: wheat and legume pre-crops

Our results show that with a small P addition to a soil with low P availability, the biomass varied among the legumes being greatest in faba bean and smallest in white lupin. The high growth and P uptake of faba bean can be attributed to its high root biomass (Table 1) compared to the other pre-crops. This is in agreement with our previous study (Mat Hassan et al. 2012b) using the same soil but a higher P supply. Further, several previous studies also reported that faba bean was able to take up more P than other legumes and wheat (Nuruzzaman et al. 2005a, b; Rose et al. 2010). This corresponds to the strong depletion of $NaHCO_3$-Pi by faba bean (Figure 1b).

White lupin had similar biomass as wheat and the lowest of P uptake among the pre-crops despite greater depletion of most P pools in its rhizosphere (Figure 2). White lupin is known for its ability to mobilise P due to the development of cluster roots which are formed under P deficiency (Neumann et al. 1999; Hocking and Jeffery 2004) and release large amounts of carboxylates. The formation of cluster roots is thought to be governed by shoot P demand rather than soil P status (Li and Liang 2005). According to Reuter and Robinson (1997), the critical shoot P concentration of white lupin is 2.0 mg kg^{-1}, hence, white lupin in this study was P deficient (Table 1). Indeed, visual observation at harvest showed that white lupin had formed cluster roots. The exudation of carboxylates as well as phosphatase enzymes by cluster roots results in mobilisation and mineralisation; which explains why most P pools, particularly NaOH-P and residual P, were depleted in the rhizosphere of white lupin (Figure 1c, e). Mobilisation of inorganic P occurs via ligand exchange (Gerke 1992) whereas organic P is mineralised by phosphatase enzymes into inorganic forms via hydrolysis (Richardson et al. 2005; Nannipieri et al. 2011). However, in the present study, organic P in the $NaHCO_3$ fraction was not mobilised but instead accumulated in the rhizosphere of white lupin. On the other hand, the concentration of NaOH-Pi and Po were lower in the rhizosphere of white lupin. This suggests that white lupin was able to mobilise NaOH-Pi and Po but this P was

apparently not taken up and instead was transformed into $NaHCO_3$-Po and microbial P. Additionally, the mobilised P could diffuse out of the rhizosphere to the bulk soil where it could be adsorbed or precipitated (Sanyal and De Datta 1991; Richardson et al. 2009).

Wheat was able to deplete residual P which is considered to be the least labile soil P form (Figure 1e) to a similar extent as white lupin. This is in agreement with previous studies which reported similar depletion of less labile P pools in legumes and non-legumes (Nuruzzaman et al. 2006; Vu et al. 2008; Rose et al. 2010; Wang et al. 2010). Although the root biomass of wheat was similar to that of white lupin, the fibrous root system of wheat would enable wheat to explore a greater soil volume (Veneklaas et al. 2003; Wang et al. 2010). Uptake of P from the soil was sufficient for wheat in the present study as its shoot P concentration was well above the critical level for wheat at this stage (2.4 mg kg^{-1}) (Reuter and Robinson 1997) (Table 1).

Chickpea was the only species that significantly depleted the very stable concentrated HCl-Pi (Tiessen and Moir 1993) compared to the unplanted soil (Figure 1d). It has been shown previously that chickpea roots excrete carboxylates (Wouterlood et al. 2004) and phosphatases (Li et al. 2003; 2004) particularly at low P supply. Our result suggests that the depletion of this stable pool by the chickpea could be due to the secretion of carboxylates which mobilised less labile P to become available in its rhizosphere. The shoot P concentration of chickpea was within the critical shoot P concentration for chickpea (2.0- 2.6 mg kg^{-1}) at this growth stage (Reuter and Robinson 1997), suggesting that P uptake was relatively low.

All pre-crops depleted the labile pools, resin P and $NaHCO_3$-Pi, confirming these pools are the main source of P uptake which is consistent with several other studies in various crops either at low or high P supply (Nuruzzaman et al. 2006; Wang et al. 2007; 2011; Li et al. 2008; Vu et al. 2008; Rose et al. 2010). This is also in agreement with our previous study in

the same soil at high P supply (Mat Hassan et al. 2011b). Thus, the labile P pools are the main source of P for wheat and legumes irrespective of levels of P supply.

Phase 2 of the rotation: wheat

The growth of the following wheat was greatest in the previously unplanted soil which could be explained by high nutrient availability particularly P which had not been taken up by a plant during the pre-crop phase. Residue addition increased wheat growth only in the chickpea pre-crop soil while it had no effect in the white lupin pre-crop soil and a negative effect in the soil pre-cropped with wheat and faba bean. The positive effect of chickpea residues on wheat growth is most likely due to release of N and P (Table 2). Nutrients would have also been released at some stage by residues of faba bean and wheat, but this positive effect was apparently smaller than the negative effect which could be due to the presence of toxic compounds or temporary immobilisation of N and P by the soil microflora during the decomposition of added residues.

The changes in the rhizosphere P pools of the following wheat were smaller than those caused by the pre-crops. Compared to the previously unplanted soil, there was an increase in the microbial P concentration in the rhizosphere of wheat following pre-crops with residue addition which can be explained by the carbon and other nutrients added with the residues which stimulated microbial growth (De Nobili et al. 2001; Mondini et al. 2006). In wheat grown in chickpea and white lupin pre-crop soil with residues, the concentration of NaOH-Po increased, suggesting formation of poorly labile organic P. This increase in microbial and organic P in pre-crop soil with residues may explain why the depletion of resin P compared to wheat grown in previously unplanted soil did not result in increased P uptake.

Without residue addition, wheat grown in faba bean pre-crop soil depleted the inorganic P fraction of resin P and $NaHCO_3$ to a greater extent than wheat in the previously unplanted soil. This suggests that wheat had a greater ability to mobilise inorganic P, However the pre-

crop faba bean also strongly depleted these P pools, to similar concentrations as those found in the rhizosphere of the following wheat. This may explain why, despite the apparent depletion of these P pools, P uptake by wheat was not increased. Chickpea and white lupin also depleted resin and $NaHCO_3$-P compared to the unplanted soil in the pre-crop phase but the concentration of these pools in the rhizosphere of the following wheat did not differ from wheat grown in the previously unplanted soil. This could be due to the differences in root biomass among the legumes. The root biomass of faba bean was greater than that of chickpea and white lupin, thus a greater soil volume was depleted by faba bean than by the other two legumes. Further, as mentioned above, P mobilised from NaOH-P by white lupin during the pre-crop phase may have diffused into the surrounding bulk soil which could still remain in labile form during the second phase. This may explain why despite a lack of depletion of the labile P pools by wheat after white lupin (Figure 2b, c), growth and P uptake were similar as in wheat after faba bean. On the other hand, the concentration of concentrated HCl-Pi was higher in the rhizosphere of wheat grown in wheat, chickpea and white lupin pre-crop soil than in wheat in the previously unplanted soil. This indicates the formation of poorly labile inorganic P which may also explain the decrease in resin P.

With residues, wheat after legumes depleted resin P and $NaHCO_3$-Pi more than wheat after wheat which can explain the higher P uptake by wheat grown in chickpea and white lupin pre-crop soil. Although the concentrations of resin P and $NaHCO_3$-Pi in wheat grown in faba bean pre-crop soil also decreased, P uptake was lower than in wheat after wheat. This can be explained by the very small root biomass and thus rhizosphere soil volume of wheat in this treatment compared to the other legume pre-crops. Only in wheat grown in faba bean and chickpea pre-crop soil there was a greater depletion of $NaHCO_3$-Po compared to wheat after wheat. This suggests a greater ability of wheat after these legumes to mineralise organic P or it may be the result of the depletion of the labile inorganic P pools assuming that there is equilibrium between labile organic and inorganic P.

Conclusions

The present study showed that white lupin can mobilise labile and less labile pools to a greater extent than the other legumes and wheat at low P supply, but this does not result in greater P uptake, possibly due to its small root biomass. In presence of residues, compared to wheat following wheat, wheat grown in legume pre-crop soil depleted labile P pools to a greater extent. However, P uptake was greater only in wheat grown in white lupin and chickpea pre-crop soil which can be explained by the greater root biomass after these two species compared to wheat grown in faba bean pre-crop soil. The greater P uptake after these two legumes appears to be due to two different mechanisms: P mobilisation in white lupin and P release from the decomposing residues in chickpea. This study showed that P uptake by a crop is only partly a function of the depletion of P in the rhizosphere; another important factor is the ability to exploit a large soil volume. More research is needed to validate the findings particularly with respect to root growth and microbial activity in both phases of the rotation. Further, employment of isotopic dilution techniques with labelled P sources would allow quantification of soil P pools being accessed by the following crops.

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Table 1: Dry weight, P and N concentrations of shoots and roots and P uptake of wheat and three grain legume pre-crops grown in an alkaline loamy sand soil with P addition at 15 mg P kg^{-1} (n=4). Values in the same column followed by different letters are significantly different ($P \leq 0.05$).

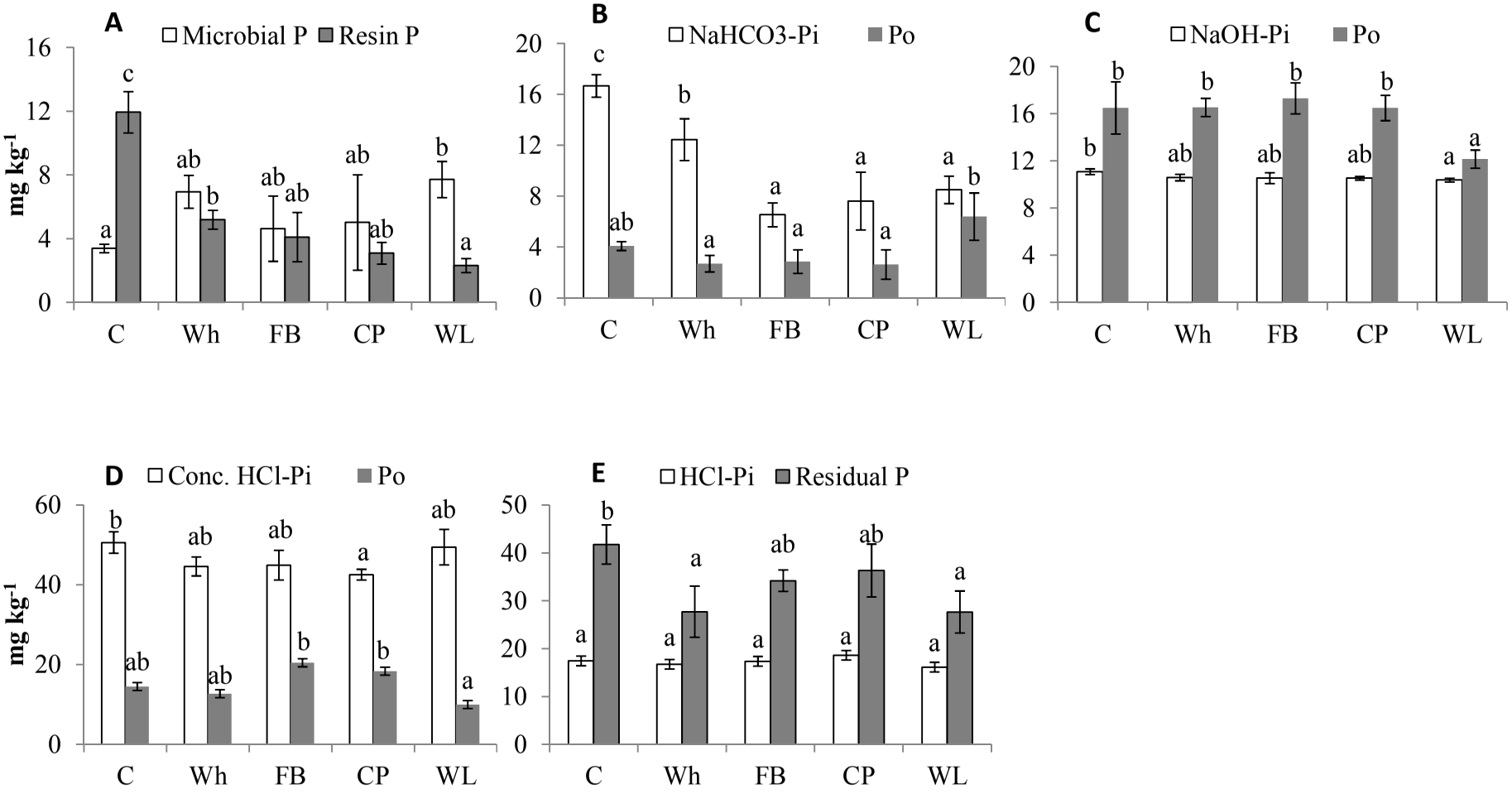
Pre-crop	Dry weight (g pot^{-1})		P concentration (mg g^{-1})		N concentration (mg g^{-1})		P uptake (mg pot^{-1})
	Shoot	Root	Shoot	Root	Shoot	Root	
Wheat	2.77 a	0.50 a	2.76 c	0.27 a	24.2 a	14.5 a	7.8 a
Faba bean	4.86 b	2.71 c	1.60 ab	2.36 c	23.4 a	35.3 b	14.5 b
Chickpea	3.35 b	1.05 b	1.36 a	1.46 b	39.3 a	36.3 b	6.1 a
White lupin	2.73 a	0.54 a	2.21 bc	2.75 c	24.6 a	40.5 b	7.9 a

Table 3: Dry weight of shoots and roots, shoot P and N concentration and P uptake of the following wheat grown for 6 weeks in the previously unplanted soil and soil previously cropped with wheat, faba bean and white lupin with and without residues (mixture of shoots and roots) (n=4). Values in the same column followed by different letters are significantly different ($P \leq 0.05$).

Wheat	Dry weight (g pot^{-1})		Shoot P concentration (mg g^{-1})	Shoot N concentration (mg g^{-1})	P uptake (mg pot^{-1})
	shoot	root			
W-Control	1.7 g	0.5 cd	2.8 a	21.5 cd	3.8 abc
Wheat					
Pre-crop	1.1 c	0.3 bcd	3.0 ab	16.0 ab	3.3 ab
Residues	0.9 b	0.3 bcd	4.5 c	17.1 abc	4.0 abc
Faba bean					
Pre-crop	1.2 cd	0.3 bcd	3.7 b	14.9 ab	4.2 abcd
Residues	0.6 a	0.1 a	4.9 c	40.6 e	2.6 a
Chickpea					
Pre-crop	1.1 cd	0.3 bcd	3.6 b	14.0 a	3.8 abc
Residues	1.6 fg	0.4 bcd	3.7 b	18.8 bcd	6.1 d
White lupin					
Pre-crop	1.4 ef	0.4 bcd	3.6 b	12.6 a	4.8 bcd
Residues	1.4 ef	0.5 cd	3.6 b	22.8 d	5.2 cd

W-Control: wheat grown in the previously unplanted soil
Pre-crop: wheat grown in pre-cropped soil without residue addition
Residues: wheat grown in pre-cropped soil with residue addition

List of figures


A
Microbial P
Resin P
mg kg-1
C
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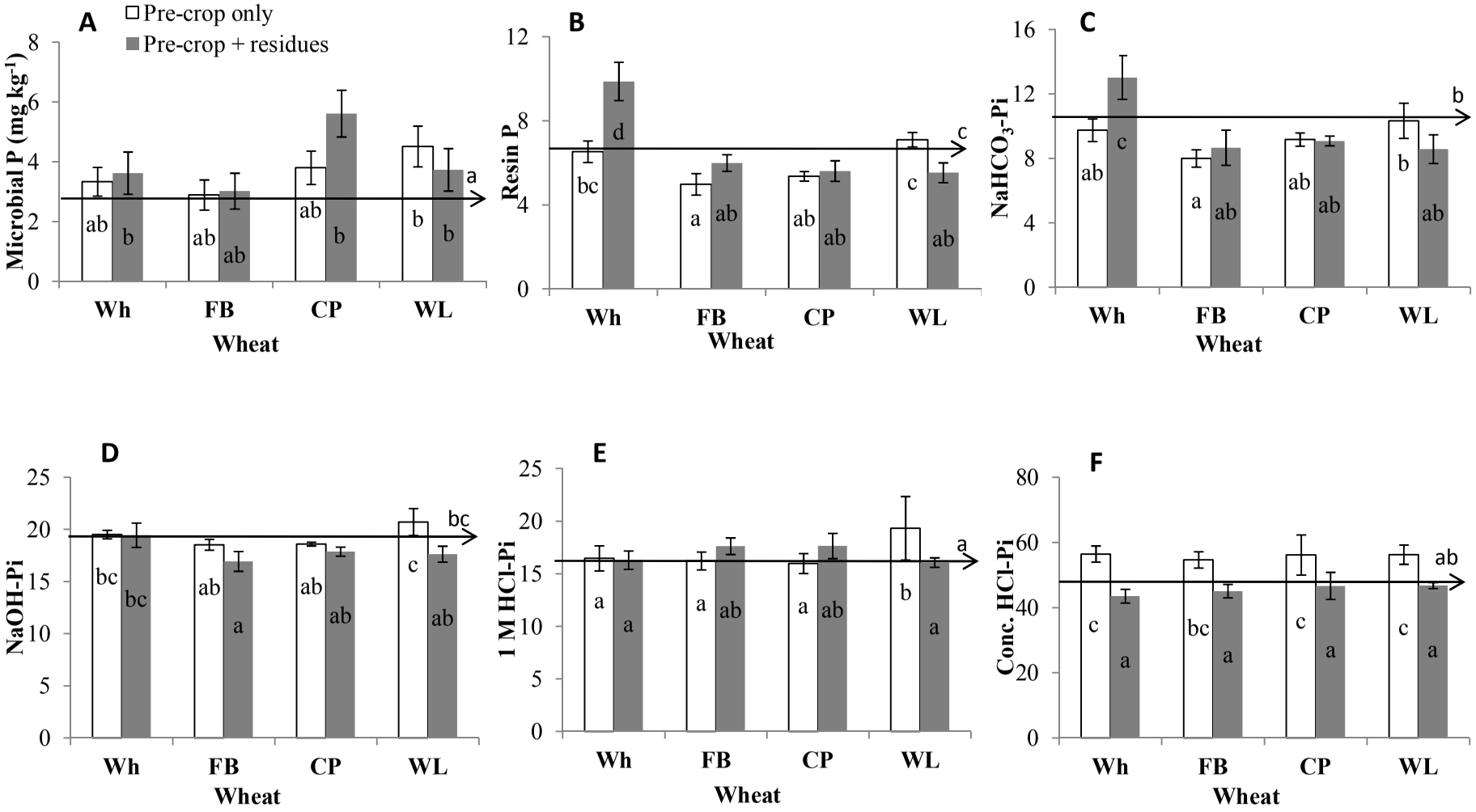
E
HCl-Pi
Residual P

Figure 1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Pre-crop only
Pre-crop + residues
Microbial P (mg kg-1)
Wh
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Wheat
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NaOH-Pi
E
1 M HCl-Pi
F
Conc. HCl-Pi

Figure 2

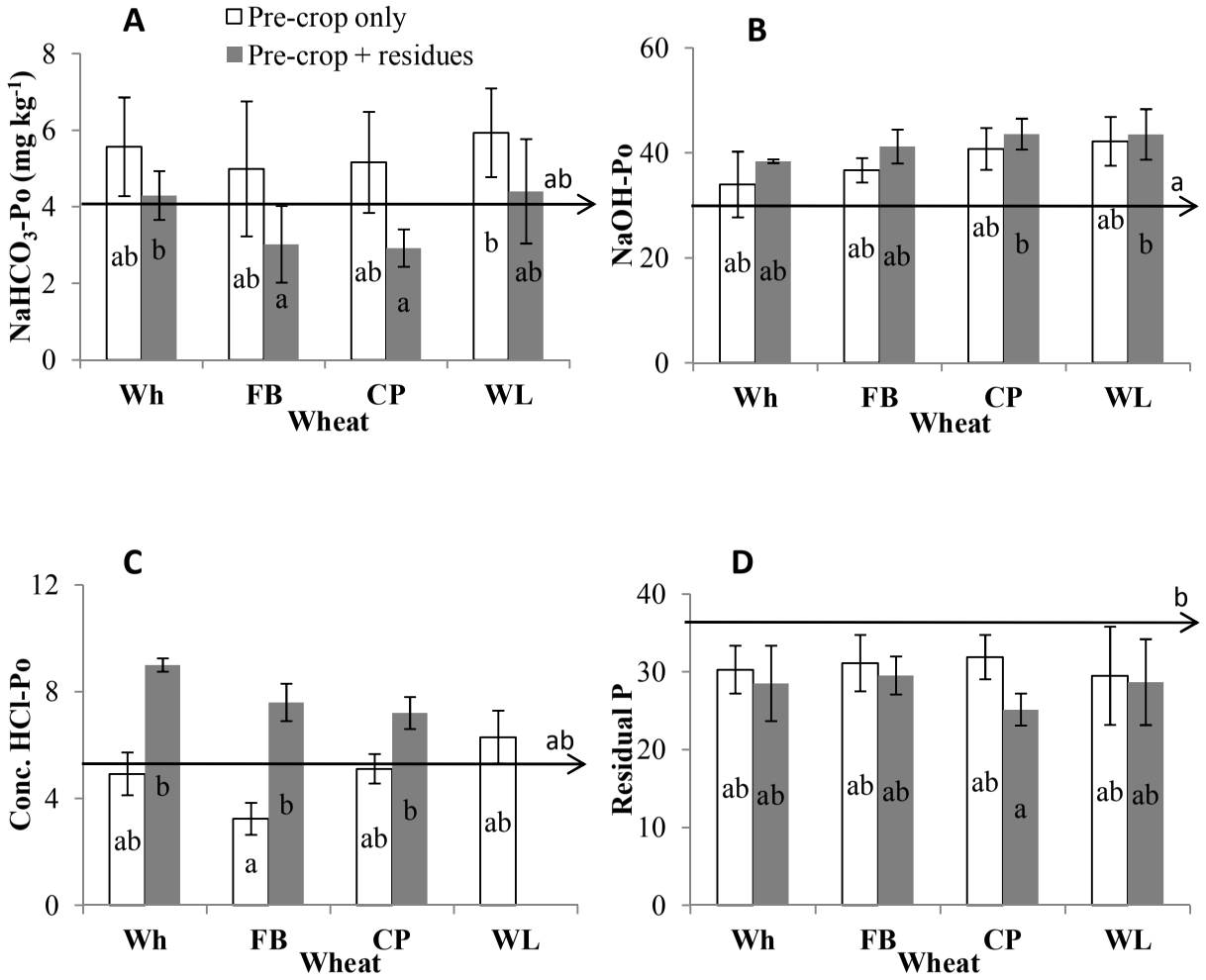
A
Pre-crop only
Pre-crop + residues
NaHCO3-Po (mg kg-1)
Wh
FB
CP
WL
Wheat
B
NaOH-Po
C
Conc. HCl-Po
D
Residual P

Figure 3

Chapter 6

Conclusions and Future Research

Conclusions and Future Research

Wheat is a major crop in Australian farming systems including in South Australia where wheat is currently grown on 2.2 million hectares of agriculture soils (Wurst 2011). Continuous cereal cropping can reduce soil fertility and consequently decrease cereal grain production. To some extent, the reduced soil fertility can be compensated for by higher fertiliser rates, however with increasing fertiliser costs and depletion of fertiliser reserves such as rock phosphate, alternative management systems are required for sustainable cropping systems. In the past 40 years, grain and pasture legumes have become significant crops with regard to their positive role in increasing cereal growth in cropping rotation. The increased growth of cereals following legumes can be explained by (i) improved N availability through N_2 fixation during the legume growth (Armstrong et al. 1997; Bagayoko et al. 2000; Dalal et al. 2004), (ii) reduction of soil-borne cereal disease, weeds and pathogens (break crop) (Reeves et al. 1984; Wildermuth et al. 1997), (iii) cycling of other important nutrients such as potassium (Rowland et al. 1986) and, (iv) of particular interest for the low P soils in large parts of Southern Australia, also increased P availability. This was shown in recent studies where N was not limiting the growth of wheat, the increased cereal growth after legumes was due to improved P availability and/or P released from the decomposing residues (Alvey et al. 2001; Kamh et al. 2002; Nuruzzaman et al. 2005a, b). However, the mechanisms behind the increased cereal growth remained unclear. Possible mechanisms include (i) mobilisation of P in the rhizosphere of the legumes which is then directly available for the following wheat, (ii) changes in the accessibility of P for wheat because legumes increased the concentration of labile P pools, and (iii) P is released from the decomposing legume residues during wheat growth. The importance of the mechanisms may vary with legume species, P level as well as soil pH. Soil pH determines which forms of P (Ca bound in alkaline soils or associated with Fe/Al in acidic soils) dominate. Therefore, the aims of the research described in this thesis

were to determine legume pre-crop effects with or without residue addition on growth, P uptake and the size of the rhizosphere P pools of the following wheat.

To optimize legume growth, P fertilisers are often applied before sowing. Based on this information, the first study was carried out by growing 5 grain legumes; faba bean, chickpea, white lupin, narrow leafed lupin and yellow lupin in an alkaline soil to which 80 mg P kg^{-1} was added. The results showed differential ability of the legumes to access soil P pools in the rhizosphere (Chapter 2). In this study, P was added to optimise growth of faba bean (based on a preliminary experiment), however the shoot P concentration of most legumes indicated deficiency except for white lupin where the shoot P concentration was adequate. White lupin had the greatest ability to mobilise labile and less labile pools but nevertheless had the lowest P uptake. This suggests that at high P availability, white lupin is capable of exuding carboxylates and phosphatases to a greater extent than the other legumes, presumably via its cluster roots. On the other hand, faba bean had the greatest P uptake but mainly depleted the most labile pools; its high P uptake can be explained by the greater root biomass compared to the other legumes. The high P uptake and its relation to the large root system of faba bean have been shown in several studies (Nuruzzaman et al. 2005a, b; Rose et al. 2010). This study led to the conclusion that legumes exhibited different mechanisms to acquire P. Nuruzzaman et al (2005a) observed positive effects in wheat following legumes with the addition of P fertiliser during the pre-crop growth. Based on this, a subsequent study (Chapter 3) was carried out to determine the effect of legume pre-crops; faba bean, chickpea and white lupin (pre-cropped soils from the experiment described in Chapter 2) with or without residue addition on growth, P uptake and changes in the rhizosphere P pools of the following wheat. A separate experiment was conducted to measure the decomposability of legumes and wheat residues. Although P was released from legume residues in the decomposition study, the addition of residues in the pre-crop soils reduced the growth of the following wheat compared to wheat grown in the previously unplanted soil and pre-crop soils without residues. The

greatest growth of wheat was achieved in the previously unplanted soil which can be explained by the fact that the nutrients that had been added before the pre-crop phase were still present as they had not been taken up during the pre-crop growth. Interestingly, the less labile pools, NaOH-Pi and Po and residual P were depleted in the rhizosphere of wheat although wheat reportedly has a low exudation of carboxylates and phosphatases (Nuruzzaman et al. 2006; Rose et al. 2010). Thus, the depletion of the less labile pools by wheat could be due to two factors (i) residual carboxylates and phosphatases from legume pre-crops maintaining at least part of their activity, and (ii) transformation of P to labile pools to gain equilibrium, and subsequent uptake by wheat. On the other hand, the depletion of these pools in the rhizosphere of wheat could also be due to P diffusion into the surrounding bulk soil. Among the legume pre-crops, faba bean had a greater effect on growth, P uptake and the size of the rhizosphere P pools than white lupin and chickpea.

The dominant form of inorganic P in the soil is strongly affected by soil pH, with Ca bound P dominating in alkaline soils and Fe/Al associated P in acidic soils. Therefore changes in P pools induced by crops may vary with soil pH. To test this, a rotation experiment was conducted in an acidic soil (Chapter 4). In this experiment, wheat was also included in the pre-crop phase to represent continuous wheat cropping. Consistent with the findings in the previous experiment in the alkaline soil (Chapter 2), faba bean had the greatest growth and P uptake among the pre-crops whereas white lupin had a lower P uptake but depleted labile and less labile P pools more strongly than faba bean. Similar to the results in Chapter 3, wheat grown in the previously unplanted soil grew better than wheat grown in soil previously cropped with legumes and wheat irrespective of residue addition. Addition of pre-crop residues reduced wheat growth but increased the size of rhizosphere P pools particularly in wheat grown after legumes. In contrast to the study described in Chapter 3, the concentration of most P pools in the rhizosphere of the following wheat increased during wheat growth which could be due to the higher rate of residue addition as well as different soil type.

It could be argued that the strong depletion of the labile P pools by most legumes found in the previous studies is due to the high P addition rate and thus P availability. To assess if this is the case, a rotation experiment was conducted in the alkaline soil used in the studies described in Chapters 2 and 3, but with lower P addition (15 mg P kg^{-1}). In agreement with the experiments at high P addition rate, faba bean had the greatest biomass and P uptake among the legumes and wheat pre-crops. On the other hand, white lupin which exhibited similar growth and P uptake to wheat, depleted most P pools including labile and less labile pools than the other pre-crops. Similar to the findings in Chapter 3 and 4, the growth of wheat grown in the previously unplanted control soil was greater compared to the pre-crop soils. However, among the pre-crop soils, P uptake of the following wheat was greatest in white lupin pre-crop soil which suggests that some of the previously mobilised P remained available to the following wheat. Chickpea as a pre-crop increased P uptake in the following wheat only in the presence of residues indicating that wheat was able to take up P released by the decomposing residues of this species. The changes in the P pools in the rhizosphere of the following wheat were modulated by the pre-crop species and the presence of their residues, but in principle similar to those found in the experiments with higher P supply, namely depletion of labile and less labile P pools.

In all studies, faba bean was the most superior species among the pre-crops in acquiring P from soil which was demonstrated by its greatest growth and P uptake regardless of P supply and soil pH. This confirms previous studies which demonstrated the adaptability of faba bean to a wide range of soil pH and conditions (Nuruzzaman et al. 2006; Rose et al. 2010; Wang et al. 2011). As mentioned above, the high P-acquisition efficiency of faba bean may be due to its extensive root system which allows it to take up more P by exploring a greater volume of soil (Singh Gahoonia and Nielsen 2004). This leads to greater P uptake although faba bean was only able to deplete the labile P pools.

On the other hand, white lupin was able to deplete labile as well as less labile P pools which can be explained by the P mobilising exudates released predominantly by its cluster roots. White lupin cluster roots exuded large amount of carboxylates particularly malate and citrate as well as phosphatases (Veneklaas et al. 2003; Nuruzzaman et al. 2006). However, despite depletion of the P pools, growth and P uptake of white lupin was lower than that of faba bean and more similar to wheat. Hence, we hypothesise that the low uptake of P by white lupin could be due to (i) diffusion of P from the rhizosphere to the surrounding bulk soil and (ii) the small root systems of white lupin which would restrict the ability to explore the soil and retrieve some of the previously mobilised P.

In all rotation experiments (Chapters 3, 4 & 5), all pre-crops reduced the growth of the following wheat compared to the previously unplanted control soil which could be due to nutrient removal by the pre-crops. In soil with higher P availability (Chapters 3 & 4), incorporation of pre-crop residues reduced the wheat growth at both low (Chapter 3) and high addition rate (Chapter 4). Apparently the addition of residues between 8 to 20 g kg^{-1} was not sufficient to increase P availability and thus compensate for the negative effects of the residues. The negative effects of residues on wheat growth include initial immobilisation of P which leads to P starvation during the early wheat growth, and the presence of toxic compounds during the decomposition of the pre-crop residues. However, in soil with low P availability (Chapter 5), the addition of pre-crop residues at 3 g kg^{-1} had little effect on the wheat growth suggesting that at this addition rate, the negative effects were negligible because only small amounts of potentially toxic compounds would be released.

For over 50 years, sequential P fractionation has been an important method to evaluate phosphorus forms in soils and sediments (Condron et al. 2005). The relative availability of these different P fractions (labile and less labile pools) varies among the soil types and properties as well as crop types (Chapter 2, 3, 4 & 5). In this study, the fractionation scheme

recovered more than 90% of the soil total P suggesting that the modified method of sequential extraction was efficient in characterizing soil P forms.

The focus of this study was the rhizosphere soil because any changes induced by plant roots will be greatest in this soil compartment and the P in the rhizosphere is the main P source for plants. However, it is also a highly dynamic soil compartment where mineralisation, adsorption and P removal occur rapidly and simultaneously. Therefore, rhizosphere soil samples taken at a given time can only provide a snapshot of the processes. Further, consistent and accurate sampling of the rhizosphere is difficult because the amount of soil adhering to the roots (the definition of the rhizosphere used in this study) is affected by soil type, water content, concentration of glues such as polysaccharides as well as root morphology, particularly root hair length (Gregory 2006; Neumann and Römheld 2002).

Suggestion for future studies

The experiments described in this thesis answered a number of questions with respect to the pre-crop effects on the growth, P uptake and the changes of rhizosphere P pools of following wheat. However, there are a number of research gaps arising from this study which could be addressed for future studies:

1. In all experiments, the contribution of P pools to the plant P uptake could not be quantified. This would be possible by addition of labelled P. The soil could be incubated for different lengths of time to allow transformations among P pools and then planted with wheat. Uptake of the labelled P and the concentration of labelled P in the different pools would help understand how P is transformed in the soil and which P pools contribute to plant P uptake. This could be accompanied by *in situ* measurement of root exudates, carboxylates and phosphatase activity to unravel the mechanisms of P mobilisation.

2. Due to the limited time of this study, the following wheat was grown for only 6 weeks in all rotations studies. The effects of pre-crops and their residues may have been different if wheat had been grown to maturity. The longer growth period would allow more P to be released from the decomposing pre-crop residues thus over-coming some of the initial negative effects of the residues found in the experiment described in this thesis. Further, a longer growth may allow wheat to more effectively exploit the soil and take up P previously mobilised by the legumes.
3. After the harvest of legumes, the soils often dry out over summer before being planted with wheat in the following autumn. Drying and rewetting of soils may have impacts on P pools and transformations and thus their accessibility to wheat. Therefore experiments should be conducted where the pre-crop soil is exposed to one or several drying and rewetting cycles before planting wheat.
4. The pot experiments used in this thesis are useful to understand mechanisms because factors such as soil water content which may affect plant growth and nutrient uptake can be held constant. However, field studies are required to determine if legumes are useful can be used in Australian cropping systems to better utilise the soil P bank and reduce P fertiliser requirement. Such field studies should be conducted in different soil types with legume species adapted to these soils to maximise their effect.

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